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The journal will consist of two parts. One part will consider previously unpublished manuscripts of original work under four main categories:

1. **Clinical or preclinical research.**
2. **Correspondence regarding manuscripts previously published in *Cancer Treatment Reports*. Letters on other topics will be published at the discretion of the Editorial Board.**
3. **Invited guest editorials and reviews. Unsolicited contributions will be considered on their individual merits.**
4. **Brief communications. Under this category the Editorial Board will consider short contributions which should not exceed five typewritten pages. Four types of communication will be considered in this category.**
   - **(a) Ideas relating to cancer treatment.**
   - **(b) Preliminary research reports.**
   - **(c) Clinical case reports.**
   - **(d) Brief clinical trials of novel treatments.**

All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board.

The second part of this journal will consist of supplements which may include proceedings of meetings and symposia and program information from the Division of Cancer Treatment, National Cancer Institute. Requests to publish information in *Cancer Treatment Reports* supplement will be considered by the Editorial Board.

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Begin the article with a short summary.

Text
Submit an original and three copies of the manuscript, typed double spaced. Each page should begin with a new paragraph. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

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Nomenclature
Names of compounds should conform to American usage. See *Chemical Abstracts* for proper nomenclature.

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Letters to the Editor can be no longer than 3 double-spaced text pages and can be submitted with no more than 1 table and 5 references; figures are usually omitted.

Symposia
Requests to publish the proceedings of symposia should be addressed to the Managing Editor. A copy of the agenda and a list of speakers should accompany such requests.

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Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, National Cancer Institute, 7910 Woodmont Ave, Rm 8C08, Landow Bldg, Bethesda, MD 20014 (301–496–6975). We invite all authors to submit with their manuscripts a short list of possible reviewers. The editoral board will take these names into consideration in selecting those who will be asked to evaluate your manuscript.

Reprints
Supplied free of charge: 250 reprints (50 reprints for Letters to the Editor) will be sent to the senior author about three weeks after publication of the article.

ANNOUNCEMENT

The readership of *Cancer Treatment Reports* undoubtedly has noticed the delay in publication of issues during the last 6 months. This is due to several factors, the major two being: (1) the managerial editorial staff has not increased during the last 7 years, while the number of papers submitted has gone from 400 per year to a projected 1000 this year, and (2) there has been a marked increase in the number of symposia that have been considered of importance to publish in *Cancer Treatment Reports*; these symposia have added to the burden of an already overworked editorial staff and helped cause a backlog of original articles.

The Division of Cancer Treatment appreciates the great deal of interest that the oncologic community has in *Cancer Treatment Reports*, and particularly appreciates the many excellent articles that have been submitted to the journal. There is currently an intense program underway to reorganize the management and production of the journal by expanding the editorial staff and negotiating a new contract with the Government Printing Office to allow for the publication of more issues per year, and thus allow for the simultaneous publication of symposia issues and issues with original articles. At the present time, the implementation of these changes is still 6 months in the future. Therefore, the Editorial Board has decided that priority will be given to the publication of issues containing original reference manuscripts. The publication of symposia will not proceed on a regular basis until a new contract has been negotiated with the Government Printing Office. In addition, a new section of the journal, **Clinical Trials Letters**, will be established. In this section, clinical trials reporting negative results may be published. Authors submitting such trials will be requested to revise their material into letters of < 300 words and these collected letters will be published periodically. This will provide the oncologic community with ready reference to studies that have been done with antineoplastic agents and treatments and will facilitate publication of topical clinical and preclinical papers in the monthly journal.

It is hoped that the efforts to reorganize the editorial office, along with the immediate changes in editorial policy, will result in a more timely publication of the journal so that in the near future *Cancer Treatment Reports* will again appear on a regular schedule.

John S. Macdonald, MD

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International Conference on Malignant Lymphomas: Current Status and Prospects
International Course on Human Immunology and Cancer Immunomodulation—A Two-Day Intensive Course
International Symposium on Nitrosoureas in Cancer Treatment
Annual Meeting of the Cell Kinetics Society

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1983.

COMMENTARY

Treatment of Small Cell Undifferentiated Carcinoma of the Lung: Update of Recent Results[1]

Raymond B. Weiss,[2,*] John D. Minna,[3] Eli Glatstein,[2] Nael Martini,[4] Daniel C. Ihde,[3] and Franco M. Muggia[2,5]

Small cell undifferentiated lung carcinoma (SCLC) accounts for about 20% of all bronchogenic cancers. In contrast to the other forms of bronchogenic carcinoma, the decade of the 1970s has seen much progress in the delineation of the natural history of SCLC as well as in its treatment. Whereas during the 1960s SCLC was almost invariably fatal (1), there are now certain groups of patients who have long survival free of disease (2), and the overall median survival has increased from about 2 months to > 1 year (3). Long-term survivors (> 2.5 years) have now become frequent enough that a case-study registry for such patients has been established, and > 106 documented cases have been entered to date (4).

These results are attributed mainly to the incorporation of effective combination chemotherapy regimens in treatment programs with or without other modalities. These programs have become increasingly aggressive and have applied effective oncologic principles such as systematic staging and thorough searches for occult metastases, intensive induction therapy, non-cross-resistant drug combinations, and radiation of the primary tumor and of "sanctuary" tumor sites such as the brain. A maximal effort has been made to increase complete remission (CR) rates and to prolong survival in an analogous fashion to what has been accomplished in the treatment of leukemia and lymphoma.

In January 1979 a 2-day conference was held at the National Cancer Institute (NCI) to update current investigations in SCLC and to encourage new directions for clinical studies. In this paper, individual rapporteurs will summarize the discussions and presentations for each therapeutic modality and staging. We shall then attempt to reach some conclusions and make suggestions for future investigations.

Chemotherapy (*J. D. Minna*)

In material submitted to this meeting and also published in abstracts in the Proceedings of American Association for Cancer Research and American Society of Clinical Oncology (AACR/ASCO) for 1979, it is obvious that there is considerable activity in the clinical investigation of chemotherapy for SCLC. For example, 44 abstracts published in the AACR/ASCO Proceedings or reported at this NCI conference dealt with SCLC, and these included 24 major chemotherapeutic investigations. The importance of achieving a CR is generally agreed upon. However, there is considerable debate about what the general treatment principles for the use of chemotherapy in SCLC are.

If one looks at the current combination chemotherapy protocols cited in the 1979 AACR/ASCO Proceedings, they include examples of nearly all the drugs found to be active as single agents. These active drugs include: cyclophosphamide (used in 16 trials), doxorubicin (Adriamycin) (14 trials), vincristine (15 trials), methotrexate (ten trials), CCNU (lomustine) (seven trials), VP-16-213 (epipodophyllotoxin) (six trials), procarbazine (six trials), high-dose methotrexate (HDMTX) with citrovorum rescue (two trials), and DTIC (dacarbazine), hexamethylmelamine, BCNU (carmustine), methyl-CCNU (semustine), and cisplatin (one trial each). There is still little objective evidence that HDMTX is active, although a group at Sidney Farber Cancer Center reports a 40% objective response rate (5). However, addition of HDMTX with citrovorum rescue to their combination chemotherapy regimen did not significantly increase the CR rate in limited- or extensive-stage disease. Investigators at Vanderbilt University also did not see a dramatic increase

[1]Sponsored by Clinical Investigations Branch, Cancer Therapy Evaluation Program (CTEP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, MD.

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in CR rate when HDMTX was added to their combination-chemotherapy regimen (6). The NCI-Veterans' Administration (VA) group[6] reported no activity of HDMTX in patients with SCLC who had relapsed with combination regimens containing low-dose methotrexate. Thus, while there may be less bone marrow toxicity from HDMTX, there is as yet little evidence that it is more useful than conventional-dose methotrexate either during induction therapy or at the time of relapse. Its role in treating or preventing central nervous system relapse of SCLC remains to be evaluated.

The Vanderbilt group reported activity of cisplatin in three of 17 patients who had been heavily treated with chemotherapy but had relapsed (7). In addition, Memorial Sloan-Kettering Cancer Center investigators have provocative evidence of a very high CR rate in a small number of patients who received cisplatin added to cyclophosphamide, doxorubicin, vincristine, and VP-16-213 for induction therapy (8). They reported that 11 of 17 patients with limited disease and six of eight patients with extensive disease entered CR; in addition, they reported converting others of these patients from a partial remission (PR) to a CR with radiotherapy, a phenomenon not readily seen in other trials. However, Eagan of the Mayo Clinic[6] found no difference in survival or response in a randomized trial comparing cyclophosphamide, doxorubicin, VP-16-213, vincristine, and chest radiotherapy with or without cisplatin.

Vanderbilt and University of Indiana investigators also reported on the activity of VP-16-213 and hexamethylmelamine, with or without procarbazine, in achieving objective responses in patients who had relapsed after receiving cyclophosphamide, doxorubicin, vincristine, and chest radiotherapy (7).[6] Whether this is a function of the VP-16-213 alone (previously shown to be active in relapsed patients) or of its combination with the other drugs remains to be seen. VP-16-213 is now routinely used in first-line therapy in many of the recent protocols. Brunner and Jungi have shown fairly dramatic schedule dependency of this drug (9). For example, a dose of 300 mg/m^2 iv once a week was inferior to a dose of 100 mg/m^2 given three times a week. This result strongly suggests that the schedule dependency of other drugs should also be investigated. In addition, studies in animal models suggest that there may be prominent differences in interactions between VP-16-213 and vincristine depending on the scheduling of the two drugs together (10).

The NCI-VA group reported[6] cytosine arabinoside (Ara-C) to be inactive since several patients in CR or PR relapsed when treated with this drug alone. In addition, Goldberg and his colleagues (11) believed there was no benefit from adding Ara-C to their combined modality treatment of cyclophosphamide, doxorubicin, methotrexate, and chest radiotherapy.

There is a general consensus that randomized trials have shown that drug combinations are better than single agents (12). In the trials reported at this meeting or at the AACR/ASCO meeting, one trial included two drugs, six had three drugs, six had four drugs, and the remainder had five, six, seven, or eight drugs. Thus, nearly all of the clinical trials involve the use of most of the known active agents. Use of multiple drugs is built in the protocols as scheduled administration rather than at the time of tumor progression. The rationale for this use of a large number of drugs apparently is based on the response rates to many of these agents after failure with primary combination chemotherapy.

The NCI-VA group (13) reported that additional patients could obtain a CR in Weeks 6–12 if they are given a combination of vincristine, doxorubicin, and procarbazine (VAP) after initial therapy with cyclophosphamide, methotrexate, and CCNU (CMC). However, randomization at 12 weeks to either continue the CMC-VAP chemotherapy or to also receive VP-16-213 and ifosfamide revealed no additional CRs and no difference in survival between the two treatments (13). Holoye of the Wood, Wisconsin VA Hospital,[6] reported that the addition of methotrexate to cyclophosphamide, doxorubicin, and vincristine (CAV) increased the CR rate in limited-stage disease in a retrospective comparison. Oldham from Vanderbilt University,[6] also in a retrospective comparison, reported that the addition of VP-16-213, procarbazine, and hexamethylmelamine (VPH) to CAV produced more responses than CAV alone. However, Einhorn[6] reported no additional CRs to VPH after CAV plus methotrexate. Dombernowsky et al (14) reported on a randomized trial of CMC plus vincristine (V) with doxorubicin and VP-16-213 (VA) at progression compared to CMCV alternating with VA. There was no difference in response rate and median survival, but there was a significant difference in duration of response in favor of the alternating combinations. Eagan[6] reported in a randomized trial no difference in overall response rate but an increase in the CRs if the arrangement of the drugs in the alternating combinations was changed: cyclophosphamide, VP-16-213, vincristine, and cisplatin alternating with doxorubicin and DTIC was superior to doxorubicin, VP-16-213, vincristine, and cisplatin alternating

[6]Unpublished observations reported at the NCI conference referred to in this paper.

with cyclophosphamide and DTIC. In summary, the data suggest that one cannot confidently predict beneficial results from adding more drugs or drug combinations to existing regimens. However, there appears to be a period in the first 12 weeks of therapy in which the addition of new drugs can increase the CR rate and the duration of the response.

Most of the reported regimens have begun with more intensive chemotherapy which later tapers off. This approach is analogous to acute leukemia treatment and was first demonstrated to be effective in a randomized trial by Cohen and colleagues (15). The next logical step is to further increase the intensity of the initial therapy. Several groups of investigators have tried this without apparent success. The NCI-VA group[6] reported no dramatic increase in the CR rate in a retrospective comparison of extensive-stage patients given four, five, or six cycles of cyclophosphamide, doxorubicin, VP-16-213, and vincristine (CAPO) in the first 8 weeks, all of which were more toxic than the prior CMC-VAP trial in a 12-week period. But if the CAPO induction cycles were cut to two, there was a dramatic falloff in the CR rate, indicating a very steep dose-response curve. In addition, they found no increase in the CR rate when various dose increases of cyclophosphamide (from 2000 to 4000 mg/m^2 in the first 6 weeks) were made in the CMC induction regimen. Abeloff et al (16) found no significant change in the CR rate when they gave increased doses of cyclophosphamide, doxorubicin, and VP-16-213 compared retrospectively to past studies. Valdivieso and colleagues (17) reported an increase in CR rate (including a 64% rate in extensive-disease patients) following treatment with an intensive, myelosuppressive combination of CAPO, but there was a high mortality rate among nonambulatory patients. Southeastern Oncology Group investigators reported that a small group of patients treated intensively with CAV experienced a high CR rate compared to prior results (18). Similarly, Holoye (*see* footnote 6) reported a higher CR rate when CAV was given in higher doses that produced more myelosuppression than in previous trials. Thus, it appears there is a very steep dose-response curve for induction therapy yielding a CR. Nonambulatory patients appear to tolerate this intensive therapy poorly. At present there is debate whether increased intensity of the already intensive induction treatment increases the CR rate. Trials having few patients report higher CR rates with increasing drug intensity, while trials with more patients indicate a plateau has been reached on the dose-response curve.

Radiotherapy (*E. Glatstein*)

In the discussion on the radiotherapeutic aspects of this cancer, several points emerged. There appeared to be uniform agreement that prophylactic cranial irradiation markedly reduced the frequency of brain metastases, but survival does not appear to be affected (19,20). It was pointed out that in order to expect any survival differences in SCLC with its marked propensity to metastasize, "prophylactic" cranial irradiation would have to be assessed separately in limited-disease patients who achieve CR. A second issue that was explicitly pointed out by Cox of the Medical College of Wisconsin was an apparent "obsession" with median survival data, rather than attention to the tail on the survival curve. Since, at the present time, the vast majority of patients with SCLC die from this disease, it is quite possible that median survival figures may not change dramatically, although significant gains may be achieved in the plateau phase of the survival curve. Median survivals cannot be expected to show improvement if the CR rate is $< 50\%$. Moreover, it was also pointed out that actuarial survival curves can be very misleading if there is an *inadequate minimum* period of followup. For this disease, when the minimum followup period is ≥ 1 year, the actuarial curve should be a very reliable projection of the ultimate outcome. However, when the data include many short-term followups, the projection could be misleading. The final point of discussion was the complexity of the interpretation of some of the chest roentgenograms in patients who receive radiation therapy. In assessing whether or not the patient has achieved CR and local control, interpretation of chest roentgenograms often proves variable among different observers. This variation is especially true when radiation changes are superimposed on the mediastinal silhouette.

A prospective study of the Radiation Therapy Oncology Group (RTOG) and the Eastern Cooperative Oncology Group (ECOG) was presented by Seydel of Thomas Jefferson University (*see* footnote 6). This study, which was restricted to limited-disease stage, included 171 patients receiving four separate treatment arms (21). All patients received radiation therapy (4500 rad) to the primary tumor and draining lymph nodes. One group of patients received no further initial treatment; a second group received cyclophosphamide and CCNU on an adjuvant basis; a third group received prophylactic cranial irradiation (3000 rad) as the only adjuvant maneuver; and a fourth group received both prophylactic cranial irradiation and the two drugs. The data were presented at this conference in a coded

form, but the difference in frequency of brain metastases following prophylactic cranial irradiation appeared to be significant. Patterns of relapse, both local and distant, appeared comparable among all treatment arms. The patients who achieved CR had a significantly longer median survival than those with PR or no response. Intrathoracic relapse appeared to be a common problem, with or without metastatic disease.

The rationale for the integration of chemotherapy and radiation therapy in SCLC treatment was reviewed by Perez of Washington University. He also outlined a new study undertaken by the Southeast Oncology Group which initially treats limited-disease patients with chemotherapy for two cycles before randomizing them to prophylactic cranial irradiation alone versus prophylactic cranial irradiation plus radiation therapy to the primary lesion. Following radiotherapy, the patients continue to receive chemotherapy. The novel aspect of this particular study is the division of the radiotherapy into three separate weekly segments of 1200 rad each, separated by cycles of chemotherapy. The purposes of this unusual approach were to: (*a*) minimize the time that patients in the study would be without systemic treatment; and (*b*) minimize, by treatment protraction, the toxicity (especially hematologic, pulmonary, and esophageal) associated with combined modality therapy.

The importance of local control and its influence on survival was emphasized by Cox (22) in a retrospective analysis of 69 patients. Thirty-five patients received conventional irradiation only, nine received local plus total-body irradiation, and 25 were treated with local-field irradiation plus chemotherapy. The interesting observations which he presented reflected the fact that, regardless of the type of therapy employed, patients whose tumor was controlled locally had improved median survival compared to those whose tumor was not controlled. For patients who received combined modality therapy, the median survival was 56 weeks when the tumor was controlled locally, but only 28 weeks in those who had local failure. Control of the local tumor had a marked influence on the survival *beyond the median*. The improvement in the median survival for patients with local control following combined modality therapy appeared to be independent of the increased survival that was associated with the introduction of chemotherapy. The importance of initiating chemotherapy as soon as initial evaluation is completed was emphasized. Cox did not present data on whether chemotherapy alone was sufficient to control bulky intrathoracic disease.

The high probability of metastatic disease in patients with SCLC was reviewed by Salazar of the University of Rochester, representing RTOG and ECOG (*see* footnote 6). Not only brain metastases but liver metastases occur clinically in approximately 30% of patients, while at autopsy disease in the liver is manifested in 60%. Consequently, the use of extended-field irradiation to cover the upper abdomen as well as the chest and brain has been advocated by some. The concept of extended-field irradiation led to investigation of upper-hemibody radiation techniques. At the University of Rochester, preliminary studies show that a single dose of 600–800 rad of upper-hemibody irradiation has achieved approximately a 16% CR and 75% PR for a total response rate that exceeds 90% (*see* footnote 6). However, Salazar emphasized that the duration of response was very short, and thus, supplementary conventional irradiation appeared desirable. In addition, the technique has been used to consolidate chemotherapy gains. In a pilot study from the RTOG and ECOG (*see* footnote 6), nine partial responders to cyclophosphamide and CCNU received 600 rad of upper-hemibody irradiation followed by 2000 rad in 1 week of conventional radiotherapy to the mediastinum. Following bone marrow recovery, chemotherapy was reinstituted. Of the nine patients who had achieved only a PR with chemotherapy, seven were converted to a CR by the hemibody irradiation followed by conventional mediastinal irradiation. These seven patients all manifested an improvement in their performance status. Four of the seven patients were alive at the time of the presentation, with three still in CR. The hematologic toxicity was not believed to be excessive. Because of the large single fraction involved in the hemibody irradiation and because of the presumed hypoxia existent in bulky mediastinal neoplasm, phase II studies with hypoxic cell sensitizers are planned by the RTOG.

Sequential hemibody irradiation, with and without hypoxic cell sensitizers, is being studied in Edmonton by Saunders and Urtasun. The study has just begun, and there were no meaningful data presented at this meeting.

Concerning the issue of mediastinal irradiation in patients with limited disease, preliminary data were discussed by Hansen, representing a group of Danish investigators (23), and Einhorn from the University of Indiana (24). In prospective studies, preliminary analysis of the effect of sequential mediastinal irradiation (which was executed somewhat differently at the institutions involved) failed to show any benefit in terms of the median survival or patterns of relapse when compared to patients who did not receive such mediastinal treatment.

Indeed, the Danish study suggested that the median survival might be shortened with mediastinal irradiation; presumably this detrimental effect was a manifestation of enhanced toxicity of combined modality treatment. Whether or not long-term survival differences occur cannot be concluded at the present time. Other investigators also have ongoing prospectively controlled studies evaluating the role of simultaneous mediastinal irradiation with chemotherapy in patients with limited disease.

Surgery (*N. Martini*)

Although in the past surgical resection was employed in patients with SCLC, few were in reality surgical candidates. In the last decade < 5% of patients with this cell type have been treated surgically.

In a comparative randomized trial conducted by the British Medical Research Council (25), 144 patients with SCLC were treated by surgery or radiation. Only three patients were alive at 5 years, with all three having received radiation therapy.

Of 368 patients with SCLC treated at Memorial Hospital from 1931 to 1961, only 7% had resectable tumors (26). Two patients were alive at 5 years, one treated by irradiation and one by resection, whereas 90% of the entire group had died within 1 year. From 1962 to 1971, an additional 292 patients with SCLC were seen (27). Although 24% were considered operable, again only 7% were resectable. Only fourteen of 292 patients had a peripheral tumor on chest roentgenogram. Whereas 65% in the earlier series received radiation therapy, nearly 92% in the more recent group were so treated. All 292 patients have since died of their disease. The overall median survival was 5 months. In the more favorable group of 20 patients with resectable tumors, the median survival was 8 months. Survival in patients treated by radiation therapy or chemotherapy was essentially the same as in the overall group.

It is early dissemination that makes local treatment ineffective in controlling this disease in any significant proportion of patients. Many investigators believe that the diagnosis of SCLC precludes successful surgical management. However, a small proportion of patients present with localized disease and may have long survival after surgical resection.

Careful staging becomes important if the benefit of surgery is to be correctly assessed. Patients with small peripheral lesions without lymph node metastases do reasonably well by resection. The survival rate in these patients in the series reported from the Armed Forces Asymptomatic Peripheral Nodule Study (28) was 36% at 5 years and was comparable to the results in patients with other histologic cell types of lung cancer.

The reason for the failure of surgery in the patient resected for cure often relates to the inability to obtain adequate staging of the systemic disease. In an autopsy study of individuals dying within 30 days of an apparent curative resection, residual disease was noted in 13 of 19 (70%) patients with SCLC (29). Of the 13 patients with residual disease, 12 had undetected systemic metastases while one had residual tumor in the thorax.

At present, chemotherapy is the major treatment for most patients. One role for the surgeon in this disease is to help establish a tissue diagnosis. Cytologic or histologic diagnosis is ordinarily obtained by sputum examination, bronchoscopy, or percutaneous lung biopsy. When there is no tissue diagnosis or the diagnosis from the material obtained is not firm, more tissue needs to be obtained by mediastinoscopy or mediastinotomy, biopsy of accessible distant metastases such as cervical nodes, or thoracotomy. Another possible role for the surgeon is after treatment. When little or no response is observed after initial induction with chemotherapy, one should raise doubt about the original diagnosis of SCLC or assume that other cell variants are also present within the same tumor and that response might have occurred only in the small cell component. Before considering any additional treatment with radiation therapy or chemotherapy, thoracotomy may be advised to correctly assess the histologic diagnosis. It is in this group of patients that one might consider partial resection to reduce the tumor burden and possibly facilitate the effectiveness of other forms of treatment.

There is also a role for surgery if the tumor presentation is that of an isolated pulmonary nodule, provided careful staging is done. Surgery is indicated if the tumor is classified as a T1N0M0 or T2N0M0 lesion. The presence of mediastinal lymph node involvement associated with a peripheral pulmonary nodule is not the type of SCLC that may benefit from surgical treatment, because positive regional node metastases reflect poor prognosis in all types of carcinoma. Whereas published British data recommend pneumonectomy on all resectable tumors (30), it is our experience that lobectomy with mediastinal lymph node dissection is generally sufficient to eradicate all visible tumor. Operative morbidity or mortality by wedge excision or lobectomy in good-risk patients is negligible compared to pneumonectomy. The more centrally placed tumors necessitate a pneumonectomy but also reflect a more advanced stage of disease. There is a direct correlation between survival and the size of the lesion at presentation, with smaller lesions associ-

ated with better survival. The location of the lesion also affects survival, with peripheral lesions having a better prognosis than central lesions (31).

In exceptional instances SCLC may be detected at an occult stage before roentgenographic evidence of carcinoma is evident. At Memorial Sloan-Kettering Cancer Center we have had one such patient with a tumor in the right-middle lobe bronchus who was treated by lobectomy.

Adjuvant systemic therapy is indicated in all patients with resected tumors in order to eradicate residual micrometastases. In the adjuvant chemotherapy trials reported by the VA Surgical Adjuvant Group (32), patients who received postresection chemotherapy appear to have done better than those who did not. Perhaps the only exception might be the occult carcinomas, such as the one described earlier, which are seen in very few patients.

Initial reductive surgical procedures prior to chemotherapy appear not to be beneficial and probably are not indicated. However, surgical excision of the primary site after a CR from appropriate chemotherapy management in a patient who originally had the disease confined to the chest should possibly be considered as a future step in the management of such patients.

Staging and Prognostic Factors (*D. C. Ihde*)

In most human malignant diseases staging procedures are performed prior to definitive therapy to assess the extent of tumor dissemination. Information gained from this process allows the choice of proper initial therapy and the assignment of prognosis for an individual patient. When published, staging data facilitate critical evaluation of the results of therapeutic trials. During therapy, repeated employment of staging procedures may identify sites of treatment failure (which may suggest alternative therapeutic strategies) and aid in determining both response to treatment and the feasibility of its discontinuation.

SCLC has traditionally been divided into limited (defined as tumor confined to one hemithorax, the mediastinum, and ipsilateral supraclavicular nodes) and extensive stages. Combination chemotherapy is presently the backbone of treatment for both stages. Whether stage-specific initial treatment (eg, chest irradiation for limited disease) is required remains undefined. At the conference session on staging and prognostic factors, discussion focused on the value of staging procedures in establishing initial prognosis and identifying sites of therapeutic failure and criteria for determination of a CR of disease, particularly when termination of therapy is contemplated. Data from the Southwest Oncology Group (SWOG), the Finsen Institute in Copenhagen, and the NCI-VA Medical Oncology Branch were presented.

The staging procedures utilized in most reported series include physical examination, chest roentgenogram, bone marrow examination, and radionuclide liver and brain scans (33,34). Radionuclide bone scans, bone radiographs, fiberoptic bronchoscopy, and liver biopsy (either percutaneously or by peritoneoscopy) are also employed (34,35). Limited disease is found after this process in approximately 30% of patients (33,34). Liver and bone are the most common sites of metastatic disease detected by staging evaluation, while tumor dissemination to bone marrow, central nervous system, and soft tissue is also frequent (34,36).

Generally accepted prognostic factors in SCLC include stage of disease and initial performance status (13); the latter factor exerts significant influence even within a single stage (33). Recent results raise the possibility that this simple two-stage staging system may be inadequate for best describing the prognostic implications of the extent of tumor dissemination.

In a small series of patients with limited disease, supraclavicular node involvement has been described as a poor prognostic sign (37). The adverse effect of tumor in supraclavicular nodes (or of superior vena caval obstruction) could not be demonstrated in the SWOG experience with a larger number of limited-disease patients (*see* footnote 6). Ipsilateral pleural effusion in this series, however, was of as grave prognostic import as the presence of other sites of extensive disease.

In extensive disease, both the SWOG and the NCI-VA group (34) found that the liver was an especially unfavorable site of metastases, possibly because of the poor response of hepatic tumor to chemotherapy compared to other sites (38). In the SWOG experience, patients with one site of extensive disease fared as poorly as those with multiple sites (36), while in the NCI-VA series (34), prognosis worsened with an increasing number of sites of involvement. In the latter group, survival of limited-disease patients was not significantly different from survival of those with a single site of distant metastases (34). In fact, after adjustment for initial performance status, no significant survival differences could be demonstrated between limited- and extensive-disease patients in the NCI-VA study.

Some of these differences may be accounted for by the aggressiveness with which the staging process was pursued, the differing definitions of whether a given site was involved, and the employment of

different treatment strategies (particularly with regard to irradiation of the primary tumor). It is possible, however, that more aggressive chemotherapy may have improved the prognosis of limited amounts of clinically detectable extrathoracic disease. This possibility could require revision of the present staging system in order to improve its clinical utility, perhaps by incorporating the number or sites of metastases or some other measure of overall tumor burden. Reporting of results by the distribution of sites of extensive disease should be encouraged in future studies.

Whether irradiation of the local chest tumor improves survival in SCLC is presently uncertain, but review of the sites of intrathoracic failure in irradiated patients may prove useful for identifying means by which the delivery of radiotherapy could be made more effective. At the University of Texas at San Antonio, sequential chest roentgenograms in irradiated patients have indicated that local recurrence frequently starts outside the radiotherapy port and is especially common with massive intrathoracic tumors (39). These data suggest the possible utility of more thorough pre-irradiation assessment of peripheral extension of the primary tumor and the employment of increased doses of irradiation for massive intrathoracic disease (39).

Serial fiberoptic bronchoscopy has proved useful during chemotherapy for identifying patients in apparent CR by radiographic evaluation who, nonetheless, had residual tumor demonstrated endoscopically and were at greatly increased risk for early progression of chest tumor (40). More recent data from the NCI-VA group indicate that the predictive value of bronchoscopic examination may be reduced in patients who have received chest irradiation, because the irradiation increases the likelihood of short-term intrathoracic disease control.

At the Finsen Institute, bronchoscopy was only one means used to seek residual disease in patients in clinical CR after 18 months of chemotherapy; bone marrow aspirate and biopsy, peritoneoscopy with liver biopsies, and occasionally mediastinoscopy were also employed (41). Fifteen of 21 patients found to be pathologically free of disease after this restaging process are alive without evidence of tumor 6+ to 25+ months after discontinuation of chemotherapy (41). Such aggressive restaging procedures could not only prove useful in deciding whether to terminate therapy in an individual patient, but also could confirm or refute whether "pathologic CRs" are being achieved by new therapeutic programs for this disease. This information might allow an earlier indication of the effectiveness of treatment.

Summary and Conclusions (*R. B. Weiss and F. M. Muggia*)

Combination drug regimens are more effective than single-agent treatments (33,42). Combination regimens produce more CRs, and these CRs translate into longer survival, re-emphasizing the need to achieve as high a rate of CR as possible. The NCI-VA group (15) has demonstrated that there is a drug dose-CR relationship. Higher overall response and CR rates were produced by an *intensive dose schedule* of a drug combination compared to more moderate doses (15). These results from a single institution are now being tested in a larger study by the ECOG.

It appears that use of two *non-cross-resistant drug combinations* early in the treatment program will achieve an even higher CR rate for certain patients. The NCI-VA group (13) has shown that two alternating combination regimens increased the CR rate from 42% to 74% in limited-disease patients. Studies to confirm this single-institution result are being conducted by several of the large cooperative groups.

In all the reports of long survival of patients treated with chemotherapy, the majority have so-called *limited disease* (2,4,41,43). Patients with *extensive disease* have lower overall response rates and CRs and shorter median survival times. Of course, limited-disease patients do better because of lesser tumor burden and possibly inherently less aggressive disease. The concept of limited versus extensive disease may be outmoded, particularly if CR to chemotherapy is the important determinant of subsequent outcome, rather than (as originally introduced) the fact that radiotherapy can encompass the known tumor. Thus, the TNM system may be a more appropriate method of reporting results. Another alternative is to classify the disease based on the sites of metastases since there are variations in the prognosis of extensive-disease patients who have, for example, liver involvement (poor prognosis) and of those who have marrow involvement (prognosis is no different from that of any other metastatic site). Tumor bulk quantitation is another factor for possible classification. Such quantitation has been shown to have prognostic value in testicular carcinoma treatment (44) and may be applicable to SCLC.

There are now appreciable numbers of patients who are *alive > 18 months* and disease-free, particularly those who have limited disease. The question arises whether to *stop treatment*. Johnson and colleagues (43), employing only four to five cycles of intensive combined modality therapy, have 50% of their limited-disease patients in unmaintained

remission for an average of 18 months. Greco et al (2) report a similar percentage. Hansen et al (41) have 24 patients from several studies who were restaged after 18 months of treatment, were found to be tumor-free, and had treatment discontinued. Fifteen of these patients remain in unmaintained remission from 6+ to 25+ months later. To be tested in a randomized study is whether maintenance therapy is necessary after a certain length of time (12 or 18 months). Proper and extensive restaging would be necessary to select those patients eligible to have therapy discontinued.

There are at least nine *active drugs* for this tumor (3). This list must be extended if further impact on survival is to be made. Phase II studies confined to this type of lung cancer are imperative. Drugs in phase II stage that are currently being tested in SCLC are AMSA, PALA, chlorozotocin, and maytansine. Zinostatin and methylglyoxal bisguanylhydrazone, both in phase II status, have not been tested yet. Phase I drugs soon to advance to phase II status are PCNU, ICRF-187, cycloleucine, and 6-diazo-5-oxo-L-norleucine (DON).

Two groups have reported on the phenomenon of *mixed histologic types*, both before and after treatment (45–47). This situation may be due to the initial presence of double primary tumors with selective ablation of the SCLC component by treatment. Alternatively, it may be attributed to therapy-induced maturation and differentiation of undifferentiated cells. This mixture of cell types or change in histology after treatment should be borne in mind when managing a patient whose tumor does not behave in the manner of most SCLC. It also reemphasizes the need for independent histologic review of material from patients involved in clinical studies.

The need for *thorough staging* is obvious. Those patients with documented disseminated disease do generally worse. If any patient is to have therapy discontinued, thorough restaging must be done to document a CR. It has been shown (40) that bronchoscopy after therapy detects occult disease, so bronchoscopy should be part of the restaging process. Peritoneoscopy also detects occult disease (35) and probably should be part of the restaging process to document CR.

The *role of radiotherapy* in this tumor is still a subject of some debate, even for patients with limited disease. Stevens and colleagues (24) have found an equivalent median survival in a randomized study comparing patients treated with combination chemotherapy versus sequential combined modalities. Some studies of combined modality treatment have still demonstrated local tumor relapse in spite of the radiotherapy (33,43,48). Tumor recurrences within the radiation port suggest that irradiation is not effective, the dosages used were inadequate, or the timing of radiation therapy and chemotherapy may be more critical than generally appreciated. Whether chemotherapy alone is as effective as combined modality treatment is a question being tested in randomized studies by several of the cooperative groups. Better local control of tumor is necessary, and new studies of different radiation fractionation schemata, variations in the timing of combined modality schedules, and adjuvant radiosensitizers may be important in helping achieve this goal.

Use of *hemibody radiation* as a chemotherapy-like treatment is the subject of new studies such as the one by Bonadonna in Milan, Italy, where it is being used in a random comparison with chemotherapy as consolidation treatment after initial chemotherapy-induced remission. If hemibody radiation effectively limits relapse in both the chest and metastatic sites, it will be a major step in improving results, particularly in extensive disease.

It has been established that *prophylactic brain irradiation* effectively limits brain involvement but does not seem to lengthen survival (3,19,20). However, a caveat is that the systemic disease must first be controlled with chemotherapy before the brain irradiation is administered "prophylactically." In a study in which the brain irradiation preceded chemotherapy, there were patients who still developed brain metastases, suggesting reseeding of the brain from uncontrolled systemic disease (49).

Another caveat is that, although brain irradiation prevents brain metastases, patients become at risk for developing *meningeal involvement* (50). The next step in prophylaxis might be the concurrent use of brain radiation therapy and intrathecal chemotherapy (51).

Lipid-soluble drugs, such as CCNU, which cross the blood-brain barrier do not prevent brain metastases (51). Other drugs, however, need to be evaluated in this regard.

Surgery is the treatment of choice for the rare patient who presents with SCLC in a solitary peripheral nodule or in an occult endobronchial lesion found by screening tests. The question of whether these patients can have their cure rate improved with postresection chemotherapy is not yet resolved but is suggested by VA Surgical Group studies (32). It should be noted that many of the long-term survivors in the NCI-VA registry had surgery as a major therapeutic modality (4).

With this tumor's known propensity for multiple histologies before and after treatment (45–47), re-

biopsy is indicated in any restaging process or when the clinical progress is not characteristic.

· The question of the value of surgical debulking of residual tumor after chemotherapy-induced remission has been raised and needs to be tested in a randomized trial of patients with limited disease.

Progress in SCLC has been substantial in the last decade, but a plateau in the prolongation of survival has been reached in most reports. Patients who are disease-free after 2 years remain a minority and are usually from the limited-disease category. Several new concepts emerging from this meeting have been outlined. Further progress may come from the identification of new active drugs, the introduction of new radiotherapy techniques and adjuvant radiosensitizers, the identification of proper use and sequencing of combined treatment modalities, and the use of new methods of eliminating disease from pharmacologic sanctuaries.

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Doxorubicin, Cyclophosphamide, and 5-Fluorouracil in Patients With Carcinoma of the Cervix or Vagina[1]

M. Steven Piver,* Joseph J. Barlow, and Jacqueline Dunbar[2]

SUMMARY

Thirty-six evaluable patients with carcinoma of the uterine cervix or vagina not amenable to surgery or radiation therapy were treated with doxorubicin, cyclophosphamide, and 5-fluorouracil; 16.5% of the patients responded, with 2.7% having a complete response. Only four (11.17%) of the 36 patients are alive, all with persistent malignancy, and none of the responders are alive. Because of the low response and survival rates, this combination is not recommended for women with recurrent cervical or vaginal carcinoma.

[Cancer Treat Rep 64:549–551, 1980]

From 1971 to 1977, we evaluated doxorubicin (Adriamycin) alone or in combination in the treatment of 100 patients with carcinoma of the cervix or vagina not amenable to surgery or radiation therapy (1). Very low response rates were obtained with doxorubicin alone (6.0%), doxorubicin and bleomycin (6.2%), doxorubicin and cyclophosphamide (0), vincristine, actinomycin D, and cyclophosphamide (0), and 5-fluorouracil (5-FU) and doxorubicin (12.5%), the last combination given over a 1-day period. For the last seven patients treated in this series with doxorubicin plus high-dose cyclophosphamide and 5-FU given over a 5-day period, a 57.1% response rate was achieved. Because of the encouraging responses in the patients receiving the high-dose cyclophosphamide, doxorubicin, and 5-FU (CAF), the study was continued. This is the report of 36 evaluable patients who received CAF for recurrent or metastatic carcinoma of the cervix not amenable to surgery or radiation therapy.

MATERIALS AND METHODS

From 1977 to 1979, 37 women with carcinoma of the uterine cervix or vagina were treated with CAF. One patient was not evaluable because she died of a pulmonary embolism during the first course of treatment. Thirty-four of the evaluable patients had disease recurrence after previous radiation therapy and two had distant metastases not amenable to surgery or radiation therapy. None of the patients had had previous chemotherapy. All patients were ambulatory and had a life expectancy of > 2 months. The primary lesion was located in the cervix in 34 patients and in the vagina in two patients. Histopathology showed squamous cell carcinoma (31 patients), adenocarcinoma (three), adenosquamous cell carcinoma (one), and anaplastic carcinoma (one). Four patients had pelvic recurrences after previous pelvic irradiation. Thirty-two women had pelvic and distant metastases or distant metastases only.

Drug doses included 60 mg/m^2 iv of doxorubicin on Day 1, 200 mg/m^2/day iv of cyclophosphamide on Days 1–5, and 200 mg/m^2/day iv of 5-FU on Days 1–5. Five patients in the latter part of the study received cyclophosphamide and 5-FU at a dose of 200 mg/m^2/day for only 4 days because of the significant bone marrow depression observed in patients in the initial part of this study. The 36 women received a total of 158 courses of CAF.

Patients were seen every 4 weeks and underwent complete physical examination, chest roentgenogram, ECG, SMA (calcium, phosphorus, uric acid, total protein, albumin, alkaline phosphatase, LDH, SGOT, blood sugar, and BUN), and cbcs. Chemo-

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TABLE 1.—Toxicity in patients treated with CAF for carcinoma of the cervix or vagina

Toxicity	No. of patients (%)
Wbcs < 1000/mm³	19 (52.7)
Wbcs < 200/mm³	8 (21.6)
Platelets < 75,000/mm³	9 (25.0)
Sepsis	9 (25.0)
Died from sepsis	2 (5.5)
Nausea and vomiting	36 (100.0)
Alopecia	36 (100.0)
ECG abnormalities	0
Cardiac toxicity	0
SMA alterations	0

therapy was repeated every 4 weeks if the wbc count was > 3000/mm³ and the platelet count was > 100,000/mm³. Doxorubicin was discontinued at a total dose of 550 mg/m².

Complete response to chemotherapy was defined as the complete disappearance of all clinical and roentgenographic evidence of malignancy for a minimum of 3 months; partial response was defined as a decrease by 50% of any measurable lesions for 3 months; no change was defined as a < 50% decrease in tumor size for 3 months; and progression was defined as an increase in the size of the lesion.

RESULTS

Toxicity

As seen in table 1, toxicity was severe; 52.7% of the patients had wbcs < 1000/mm³ and 21.6% had wbcs < 200/mm³. Moreover, 25% developed sepsis concomitant with severe leukopenia, 5.5% of whom died from sepsis. Because of severe leukopenia and/or thrombocytopenia, the dose of cyclophosphamide and 5-FU was reduced by 20% for 13 (36.0%) of the women, and by an additional 20% for two of these women.

Response

As seen in table 2, one (2.7%) patient had a complete response and five (13.8%) patients had a partial response; the total response rate was 16.5%. Twelve (33.3%) patients had stationary disease; 11 of these women subsequently developed disease progression. A total of 18 patients had progression of their disease.

TABLE 2.—Responses of patients treated with CAF for carcinoma of the cervix or vagina

Response	No. of patients (%)	Duration of response (mos)
Complete	1 (2.7)	18
Partial	5 (13.8)	3–9
Stationary disease	12 (33.3)	3–12
Disease progression	18 (50.0)	—

Survival

None of the responders are alive; five died from their malignancy, and one died from complications of therapy. The one complete responder survived 24 months, and the patients with partial response survived 3, 6, 8, 9, and 17 months, respectively, with a median duration of survival of 8.5 months. Patients with stable disease survived a median of 7 months (range, 4–26); nine of these 12 died of their disease. The median duration of survival for those patients with disease progression was 3 months (range, 1–7); 16 of these women died of cancer, one died from complications of treatment, and one remains alive with cancer. Thus, only four (11.1%) patients remain alive, all with cancer. The median duration of survival for the 36 patients was only 4.5 months.

DISCUSSION

Of the 36 evaluable patients treated with CAF for cervical or vaginal cancer not amenable to surgery or radiation therapy, there was an overall response rate of 16.5% and a complete response rate of 2.7% (one patient). Moreover, the median duration of survival for the 36 women was only 4.5 months: 8.5 months for those responding to therapy, 7 months for those with stationary disease, and 3 months for those with disease progression. Nineteen (52.7%) patients developed wbc counts of < 1000/mm³ and eight (21.5%) patients developed wbc counts of < 200/mm³. Nine (25%) of the women developed sepsis at the time of severe leukopenia and two subsequently died from this complication.

It is concluded that the overall and complete response rates and the duration of survival are too low and the complication rate is too high to warrant the continuation of this combination chemotherapy in women with carcinoma of the cervix or vagina

not amenable to surgery or radiation therapy. Because of the reported activity of *cis*-dichlorodiammineplatinum(II) in patients with squamous cell carcinoma of the cervix (2), we are now evaluating cyclophosphamide, doxorubicin, and *cis*-dichlorodiammineplatinum(II) in the treatment of women with recurrent cervical or vaginal carcinoma.

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Probable Cure of Metastatic Testicular Tumors Treated With Sequential Therapy[1,2]

James A. Neidhart,* Richard Memo, Earl N. Metz, and Henry Wise[3,4]

SUMMARY

This clinical trial explores the possibility of inducing long-term unmaintained complete remissions in testicular cancer using sequential multimodality therapy. We designed a prospective study using a combination of vinblastine and bleomycin for three courses followed by *cis*-dichlorodiammineplatinum(II), actinomycin D, and vincristine until a surgically proven complete remission was achieved. One to three additional consolidation courses were given following surgery, and then all therapy was stopped. Eleven of 15 patients completing therapy have achieved complete remission, with seven patients remaining free of disease without maintenance therapy for 15–36 months (average, > 27 months). We conclude that patients with disseminated nonseminomatous testicular cancer should be treated for cure with intensive primary therapy. Prolonged disease-free status can be achieved without maintenance therapy, and the value of maintenance therapy needs further study.

[Cancer Treat Rep 64:553–558, 1980]

While seminomas of the testis are curable by radiation therapy, nonseminomatous testicular tumors have usually been fatal and account for 11%–13% of cancer deaths in 15–34-year-old males (1). The development of *cis*-dichlorodiammineplatinum(II) (cisplatin) and its use in combination chemotherapy has made it possible to induce complete disappearance of tumor in the majority of patients with far-advanced nonseminomatous testicular cancer. Most treatment regimens which have produced long-lasting remissions have included maintenance therapy. Maintenance therapy, however, is not necessary to obtain prolonged complete remissions in other highly treatable malignant diseases such as gestational choriocarcinoma (2), Hodgkin's disease (3), and acute lymphoblastic leukemia in children (4). The present study shows that it is possible to obtain prolonged complete remission in nonseminomatous testicular cancers with intensive primary chemotherapy and surgery, without maintenance therapy. The presence of choriocarcinoma or elevated levels of human chorionic gonadotropin (HCG) in our patients decreased the chances of achieving a long-lasting complete remission.

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MATERIALS AND METHODS

Eighteen patients with far-advanced nonseminomatous testicular cancer have entered the program since March 1976. Fifteen patients have completed treatment and constitute the basis of this report. The patients ranged from 16 to 55 years of age, with a median of 23 years. Table 1 shows the extent of disease. All patients had extensive, nonresectable, nonseminomatous testicular cancer with clinically measurable disease. The disease was

TABLE 1.—Extent of tumor in study group

Disease site	No. of patients
Stage IIc disease	
Abdomen alone	4
Stage III disease	
Lungs alone	3
Abdomen plus—	
Lungs	3
Lungs and nodes	2
Lungs and liver	1
Liver	1
Skin (scrotal recurrence)	1
Lungs and liver	1

TABLE 2.—Correlations of serum levels of HCG to histologic classification

Histology	No. of patients	No. of patients with elevated HCG
Pure		
Embryonal carcinoma	7	1
Choriocarcinoma	2	2
Mixed		
Embryonal carcinoma plus—		
Seminoma	2	0
Teratocarcinoma	1	0
Choriocarcinoma plus—		
Embryonal carcinoma	2	1
and seminoma	1	0

stage III (spread of tumor above the diaphragm) in 11 patients and stage IIc (gross residual retroperitoneal disease) in four patients, according to the Testicular Tumor Study Group Staging System (5). The four patients with abdominal disease had only unresectable tumors with measurable postoperative tumor ranging from 3 × 6 to 8 × 9 cm in size. One patient with pulmonary involvement had only a 5 × 5-cm nodule while the other patient had several nodules < 2 × 3 cm in size.

The most consistent and marked laboratory abnormality was LDH elevation in 12 patients, with levels from 521 to 6830 units/dl and a mean of 2328 units/dl (normal, < 450 units/dl). The carcinoembryonic antigen level was 660 ng/dl in one patient with yolk sac elements present in his tumor, but assays for carcinoembryonic antigen were negative in six other patients tested. Table 2 shows the correlation between HCG levels and the presence of choriocarcinoma in histologic sections. Only one patient had an elevated level of HCG without a pathologic diagnosis of choriocarcinoma. Alpha-fetoprotein (AFP) was initially elevated in one of seven patients tested. Six patients had elevations of liver enzymes that did not necessarily correlate with proven hepatic metastases. Ten patients had received prior therapy: two patients had received radiation therapy only, one had received chemotherapy only, five had received both modalities, and two had received radical node dissection. All patients were fully informed of the experimental nature, risks, and potential benefit of the treatment and signed informed consents prior to therapy.

A complete history and physical examination, chest roentgenogram, and analyses for tumor markers (LDH and HCG), liver enzymes, and creatinine clearance were performed prior to each treatment. Whole-lung tomography was required to document the absence of pulmonary disease if the chest roentgenogram was negative. Abdominal echograms and liver scans were initially obtained and were done at least every 2 months if abnormal. All patients were seen monthly for 1 year following therapy, and then every 2 months. The β-subunit of HCG was measured by radioimmunoassay as described by Vaitukaitis et al (6) using an antisera that cross-reacted < 5% with human pituitary luteinizing hormone.

The chemotherapy consisted of sequential combination regimens designed to use the optimum doses of the most active single agents. Table 3 shows the courses and doses of drugs given for both regimens. The first course consisted of 4 mg/m² of vinblastine on Days 1 and 2 repeated every 21 days for three courses given with 15 units/m² of bleomycin twice weekly for 7 weeks. Patients were able to receive the full projected doses of this regimen with few delays. All patients then entered the second arm consisting of 20 mg/m² of cisplatin on Days 1–5, 0.3 mg/m² of actinomycin D on Days 1–4, and 1.4 mg/m² of vincristine (maximum, 2.0 mg/m²) on Day 1 repeated every 3–4 weeks as bone marrow recovery permitted. The cisplatin was given with parenteral hydration, furosemide, and mannitol diuresis. Dose reductions for renal toxicity were directly proportional to the reduction in creatinine clearance from the previous treatment. When patients had achieved either clinical complete remission or a partial response stable for 2 months, a radical retroperitoneal node dissection as described by Staubitz et al (7) was performed. Residual pulmonary disease was resected if feasible. The surgical procedures were planned both for potential therapeutic benefit and as confirmation of complete response prior to stopping chemotherapy. Postoperatively, patients received one to three courses of the second regimen to complete four "consolidation

TABLE 3.—Courses and doses of drugs

Drug	No. of courses administered		Total dose given (mg/m²)	
	Range	Mean	Range	Mean
Regimen 1				
Vinblastine	2–4	2.7	14–34	22.8
Bleomycin	9–16	13.5	135–370	230
Regimen 2				
Cisplatin	2–9	6.3	200–905	522
Actinomycin D	4–9	5.9	3.6–11.3	6.3
Vincristine	3–9	5.6	3–10	5.6

courses" after clinically documented complete remission. Patients received an average of four courses (range, one to six) of regimen two preoperatively, and an average of 6.3 courses (range, two to nine) overall. Eighty percent of the projected doses of cisplatin and actinomycin D were given with short delays in approximately one third of the courses due to prolonged myelosuppression or patient intolerance of nausea and vomiting. The average time between courses was 3.8 weeks.

A complete remission (CR) was defined as the disappearance of all clinical evidence of tumor, confirmed by surgery if possible, and lasting at least 1 month. Patients whose only clinical evidence of tumor was shown at surgery to be nonmalignant were considered to have achieved a CR while receiving chemotherapy. Normalization of abnormal HCG, AFP, and LDH were required for a CR. A partial remission (PR) was defined as the reduction of all measurable tumor by > 50% for at least 1 month. Progressive disease was defined as any increase in tumor size of > 25% or the appearance of a new lesion.

RESULTS

Eleven of the 15 patients (73%) attained CR. Three patients achieved a CR during treatment with the first regimen, seven achieved a CR during treatment with the second regimen, and one achieved a CR only after surgical resection of residual choriocarcinoma. Three patients achieved a PR for an overall response rate of 93%. There was no obvious relationship of response or cure to treatment intervals or doses, but all patients were intensively treated (table 3). The only patient who failed to respond was a 55-year-old man who developed acute respiratory insufficiency and died at home after receiving 210 ùnits/m² of bleomycin. Chest roentgenogram indicated that his extensive disease had been stable, but an abdominal echogram done shortly before death showed progression. Although neither patient with pure choriocarcinoma achieved CR, the presence of choriocarcinoma in a mixed tumor did not seem to affect the chances for a CR (table 4). The PR in one patient with pure choriocarcinoma lasted only 8 weeks and was followed by rapid progression and death.

TABLE 4.—Response and relapse related to histologic type and HCG levels

	Total No. of patients	No. of patients with—				Duration of CR (mos)
		CR	PR	Progressive disease	Relapse from CR	
Overall response	15	11	3	1	4	
Histologic type						
Pure						
Choriocarcinoma	2	0	2	0	0	—
Embryonal	7	6	0	1	1	41+,39+,30+,23+,19+,10
Teratocarcinoma	1	1	0	0	0	39+
Mixed						
With choriocarcinoma	3	3	0	0	3	9,8,1
Without choriocarcinoma	2	1	1	0	0	42+
HCG						
Positive	4	1	2	1	1	9
Negative	11	10	1	0	3	42+,41+,39+,39+,30+, 23+,19+,1,8,10

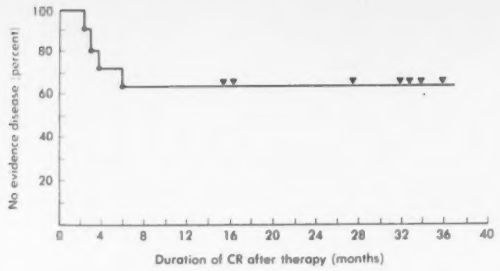

FIGURE 1.—Disease-free interval after cessation of all therapy for 11 patients entering CR, updated to October 1979. Arrows = patients at risk.

Surgery was of considerable help in defining the absence of malignant tumor after induction chemotherapy. Eight patients had achieved considerable reduction in tumor size but failed to achieve clinical CR after two to three courses of the cisplatin-containing combination of chemotherapy. Two patients had positive chest roentgenograms and six had positive abdominal echograms. Six of these patients were found to have mature teratomas (four), necrotic tumors (one), or fibrosis (one) on surgical exploration, with no evidence of viable tumor. Only two of these patients have subsequently relapsed. The only patient in whom malignancy was resected was in clinical CR before radical node dissection and relapsed 9 months after surgery. Two patients in PR had unresectable tumor but continued receiving chemotherapy. One patient remains alive with disease at 6 months and one died 14 months after surgery. Surgery was not performed in four patients because of early death (two), refusal (one), and extensive radiation fibrosis (one).

The durations of CRs are shown from start of CR in table 4 and from cessation of all therapy in figure 1. The four patients who relapsed have done so within 10 months of entering CR and within 6 months of stopping therapy. All four patients who relapsed had either choriocarcinoma or elevated HCG at relapse. The difference between the one of eight relapses in patients with no initial evidence of choriocarcinoma and the three of three relapses in patients with choriocarcinoma is significant ($P < 0.01$, Fisher's exact test). During re-treatment, one relapsed patient has entered a second CR and two have entered a second PR.

The most distressing toxic effects for patients, and often for physicians also, were related to the cisplatin regimen and consisted of marked nausea and vomiting, weight loss, and fatigue between therapy. Two patients absolutely refused to continue cisplatin beyond two courses because of vomiting. Bleomycin may have had a pulmonary toxic effect on the patient who died during the first course. Two other patients developed pulmonary symptoms and four developed mild abnormalities of pulmonary function. Seven patients had skin changes from bleomycin and one developed Raynaud's phenomenon. While all patients had evidence of bone marrow suppression, leukopenia of < 1000 cells/mm^3 developed in only nine of > 130 courses and infection occurred only once. A decrease in creatinine clearance of > 25% occurred on nine occasions and resulted in reduction in dose of cisplatin six times. The combination containing cisplatin and vincristine produced peripheral neuropathy in seven patients. A high-frequency hearing loss developed in three patients. All patients completing therapy are fully functional and have gained considerable weight after treatment. The only bothersome residual side effect is retrograde ejaculation in all patients who had radical node dissection. Psychologic support and reassurance is, however, a critical ingredient of this regimen.

DISCUSSION

Slightly over a decade ago, actinomycin D alone or in combination was the best available therapy for metastatic nonseminomatous cancer. Mackenzie (8) reviewed 154 patients of whom 24 (15%) had clinical disappearance of disease and 13 (8%) remained free of disease with maintenance therapy for an average of 43 months. Long-term, disease-free remission was possible, but it was infrequent and seemed to require maintenance chemotherapy. Bosl et al (9) recently reported that 11 of 13 patients who had achieved a CR while receiving mithramycin remained free of disease without maintenance therapy for 43 to 184+ months. More recently, vinblastine and bleomycin combinations have produced CR rates of about 30%–45% (10,11). CRs could be maintained with vinblastine for > 2 years in approximately half the patients. With the demonstration that an investigational drug, cisplatin, had significant activity in these tumors, the door opened to use of a variety of promising combinations of agents.

Perhaps the most enthusiastic reports in terms of long-lasting CRs are those of Einhorn and Donohue (12) and Einhorn and Williams (13). Cisplatin was added to vinblastine and bleomycin as induction therapy followed by maintenance therapy with vinblastine and bacillus Calmette-Guérin for 2 years. Of 47 evaluable patients, 33 (70%) initially achieved a CR with drugs alone; after surgical re-

section of residual disease 38 (80%) achieved CR. This study was updated in 1979 with 28 (60%) patients alive and disease-free with a minimum followup of 28 months (13). The number of these patients who will remain disease-free after cessation of maintenance therapy should be known in a year or so. Samson and Stephens (14) have reported a 56% CR rate in 57 patients treated with a combination of drugs similar to that used by Einhorn for induction, followed by maintenance therapy with vinblastine, chlorambucil, and actinomycin D. Three patients have relapsed while receiving maintenance therapy although the median followup of 20 weeks was too short to draw conclusions regarding duration of CR. Cvitkovic et al (15) have obtained CR in 18 of 27 patients treated with a combination of bleomycin, vinblastine, cyclophosphamide, actinomycin D, and a single injection of high-dose (120 mg/m^2) cisplatin. This program includes maintenance therapy with vinblastine, chlorambucil, and doxorubicin as well as a re-induction at 5-month intervals with the cisplatin combination. We are not aware of a published long-term followup on these patients. Maintenance therapy continues to be a common ingredient of these programs. Stoter et al (16) recently reported results similar to those of Einhorn et al with a regimen that includes maintenance therapy with vinblastine and cisplatin for 2 years. The 73% CR rate seen in our patients with advanced disease compares favorably to that seen with a variety of other platinum-containing combinations. Although an improved CR rate should be sought, an equally important question is whether duration of CR is dependent on the initial treatment program and if maintenance therapy is a necessary ingredient for potential cure.

The present program was designed to accomplish tumor reduction with a combination of drugs (vinblastine and bleomycin) known to induce short-term responses in a large percentage of patients. This was followed with cisplatin and actinomycin D at maximum tolerable dose and schedule with the hope that eradication of minimal residual disease would produce cure and negate the need for maintenance therapy. Randomized studies in Hodgkin's disease (3) and nonrandomized trials in childhood acute leukemia (4) suggest that maintenance therapy does not necessarily improve survival or decrease the relapse rate after intensive initial treatment. Prolongation of CR with maintenance therapy may result from slowing of tumor growth without affecting overall recurrence or survival and may not warrant the discomfort, cost, and risk of prolonged chemotherapy. The results reported here offer evidence that prolonged CR and probably cure are possible without maintenance therapy. Prospective trials of several induction regimens with and without maintenance therapy should provide a definite answer.

Surgical resection of residual disease after chemotherapy was originally included for its potential therapeutic benefit. The major value of surgery, however, proved to be in the accurate assessment of clinical response. Eight patients had residual clinical evidence of disease following chemotherapy and might have been changed to other treatments as failures without histologic proof of benign disease. The only patient who had surgical resection of residual tumor has relapsed. Radical reductive surgery is being used as primary therapy of metastatic testicular cancer. Merrin et al (17) have resected abdominal and thoracic metastases as reductive surgery prior to chemotherapy with cisplatin-containing drug combinations in 37 patients. Sixteen of these patients are alive and disease-free receiving maintenance therapy for an average of 12.9 months. These results are not substantially different from those reported here despite the fact that only one of our patients had cancer-reductive surgery. We continue to use surgical staging after chemotherapy in most patients and believe that the value of initial reductive surgery in patients with advanced disease needs clarification.

The significance of elevated HCG levels remains unclear. Hobson (18) correlated elevated titers of HCG to poor prognosis and felt these elevations were due to the presence of chorionic tissue perhaps missed in sectioning tumors. Mostofi (1), on the other hand, does not feel that elevated HCG levels per se warrant a diagnosis of choriocarcinoma. Each patient who has relapsed from CR in our study has had either elevated HCG titers or choriocarcinoma on histologic section. It is possible that clinical CR in mixed tumors may largely reflect the disappearance of nonchoriocarcinomatous elements of the tumor since the two patients in this study with pure choriocarcinoma failed to achieve CR. Mackenzie (8) did have two of five patients with choriocarcinoma who remained free of recurrent disease 24 and 85 months after starting treatment with actinomycin D, chlorambucil, and methotrexate. The presence of choriocarcinoma may demand additional or modified therapy if the goal of treatment is cure.

It seems clear that CRs can frequently be achieved with a variety of regimens in metastatic germ cell tumors and should be the minimal goal of therapy. It is likely that cure of nonseminomatous testicular tumors—particularly those without chorionic components—is now a reasonable goal of therapy. Considerable patient support is necessary to assure optimum initial treatment. The potential value of

maintenance therapy and the role of reductive surgery must be evaluated in larger randomized trials but neither are necessary to achieve cure.

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L-Phenylalanine Mustard (L-PAM): The First 25 Years[1,2]

Raymond L. Furner* and Robert K. Brown[3]

HISTORY: SYNTHESIS AND ACTIVITY

L-Phenylalanine mustard (L-PAM) is a derivative of *nor*-nitrogen mustard, an alkylating agent. L-PAM was synthesized by Bergel and Stock (9) in 1953 with the hope that the incorporation of phenylalanine, a precursor of melanin, into the molecule would give the compound some selectivity of action against melanoma. To date there is no convincing evidence that the phenylalanine group contributes any directive influence toward an amino acid metabolizing site, and the desirable therapeutic activity probably results from the chemical and pharmacologic properties of the compound as a whole. Almost simultaneously with the work of Bergel and Stock, Larionov and his co-workers (38) synthesized sarcolysin. Careful work by Schmidt et al (59), indicated that the L form was approximately twice as active as the D form, and so L-PAM has become the isomer of choice.

Although L-PAM was initially used alone against multiple myeloma (10), subsequent investigations indicated that it might be more effective in combination with prednisone (18). Since that time, L-PAM has proved useful in the treatment of patients with cancer of the ovary (54) and testes (66) and as an adjuvant to surgery in the management of primary breast cancer (20).

The management of primary breast cancer is of particular significance. Continuous oral administration of L-PAM (0.15 mg/kg/day for 5 consecutive days every 6 weeks) as an adjuvant to mastectomy in the management of patients with primary breast cancer and pathologically active auxiliary nodes resulted in a significant increase in the length of the disease-free interval in premenopausal women (20). The report was timely because it illustrated the therapeutic potential of combined modalities of treatment. It also demonstrated that more information is needed concerning the mechanism of action of this useful agent. The original studies have been extended (21) to include the use of 5-fluorouracil in combination with L-PAM with promising results. This was the first hard data to demonstrate that surgery followed by aggressive chemotherapy was better than surgery alone in the treatment of neoplastic disease. Based upon preliminary clinical data plus animal data that will be discussed below, L-PAM may be useful against advanced cancer of the biliary and gastrointestinal tracts (12). The effectiveness of L-PAM against murine tumors has been demonstrated in a variety of experimental systems (17,26,49,51,52,55,56). In addition, the drug has demonstrated a synergistic effect with doxorubicin against advanced L1210 leukemia in mice.

In clinical situations for the treatment of multiple myeloma, favorable results have been demonstrated with continuous oral administration of L-PAM (3,10,25,32,41). With L-PAM alone the rate of objective response was approximately 4%, but in combination with prednisone the rate was 74% (25).

METHODS OF ANALYSIS

Although L-PAM has been available for > 20 years and has demonstrated clinical usefulness, only a few studies have attempted to determine its metabolic fate or to ascertain its specific mechanism of action. For the most part studies have been hampered by the lability of the compound and lack of specific analytic techniques. The earliest studies were conducted by Cohn (16) and used ^{14}C-labeled sarcolysin to study the distribution of radioactivity in tissues from rats. He reported levels of radioactivity that were similar in liver, tumor, and blood but were at least three times higher in the kidney. He reported too, that the supernatant fraction and the microsomes from the kidney had the highest specific radioactivity levels and that the proteins always had a higher specific radioactivity than the nucleic acids separated from the same tissue. Unfortunately, Cohn was not able to identify the materials found in the various tissues or to determine

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whether they were true metabolites or merely products of hydrolysis.

A colorimetric method which required the use of nitrobenzylpyridine and was specific for alkylating activity was introduced (34) for the analysis of alkylating agents in blood and tissue. Unfortunately, the method was sensitive to both mono- and bi-functional alkylating agents, so that generation of the proper color occurred with L-PAM as well as the monohydroxy hydrolysis product of L-PAM which still possessed a single alkylating group. Not only did the assay lack specificity, but it also lacked sensitivity. The procedure was capable of determining alkylating activity at concentrations in excess of 5 μg/ml of plasma, but the therapeutic levels of the compound were in the nanogram per milliliter range.

In an effort to improve upon the sensitivity required for an L-PAM assay, Chirigos and Mead (15) used differential fluorometric analysis to investigate L-PAM and its hydrolysis products. However, they found that interference by various tissue constituents and by the products themselves made fluorometric analysis impractical.

Gorgas et al (27) developed a gas chromatographic method that required derivatization with BSA trimethylsilyl. The technique was excellent in terms of specificity and sensitivity for dosage formulations, but it was never adapted to biologic systems.

Thus, the major obstacle to the generation of biologic information about L-PAM was the lack of a method that was sensitive, specific, and easy to use in biologic systems. Those requirements were met in our laboratory (23,24) through the use of high-pressure liquid chromatography (HPLC). The method met the basic requirements for a sensitive and specific analysis of a nonradioactive material and provided: (*a*) stability of the parent compound for several months; (*b*) a solvent for the sample that was compatible with initial conditions on the liquid chromatograph; and (*c*) a sample that was > 95% protein-free when injected onto the column.

Analytic separation was accomplished using a liquid chromatograph with two pumps, a solvent flow programmer, a μBondapak C_{18} Column, and a UV detector. Using this technique, unlabeled L-PAM and a monohydroxy product, L-MOH (4-[(2-chloroethyl)(2-hydroxyethyl)amino]-L-phenylalanine), were identifiable in extracts of biologic samples. When ^{14}C-L-PAM was used, fractions of the effluent were collected and assayed for radioactivity. The results indicated that ^{14}C-L-PAM was converted to both products of hydrolysis and products of metabolism. Subsequent to our work, Chang et al (13) reported the development of a similar method which required isocratic conditions and a solvent consisting of methanol and acetic acid. The two methods were complementary in that they allowed independent confirmation of results related to parent compound, even though the isocratic method was not capable of rapid and quantitative separation of the products of metabolism and/or hydrolysis.

The method was applicable to all tissues studied including whole blood and was limited only by the sample size available for injections up to 2 ml.

CHEMISTRY

The chemistry of L-PAM proved difficult to study without a specific and sensitive assay and, as a result, much of the chemistry has been developed in parallel with the development of analytic methodology.

It had long been known that L-PAM was hydrolyzed in aqueous solution to other products. Cohn (16) was aware of this as were Chirigos and Mead (15), but they could not identify the products. However, using HPLC as described above, we were able to collect fractions and identify the compounds represented as L-PAM, L-MOH, and L-DOH (4-[bis(2-hydroxyethy!)amino]-L-phenylalanine), respectively, by means of mass spectrometry (23).

Solutions of L-PAM (100 mg/ml) kept on ice showed no appreciable change in composition over a 4-hour period, regardless of whether the solvent was phosphate buffer, monkey serum, or 11 mM HCl. However, solutions of L-PAM (1 mg/ml in 11 mM HCl) refrigerated at 4°C for several days showed a substantial decrease in L-PAM (43%) and a corresponding increase in L-MOH. Increasing the acidity retarded the decomposition as did freezing. Hydrolysis was more rapid at 37°C, with a rate of about 50%/hour during the first 30 minutes and about 30%/hour thereafter. Depending upon the concentration of L-PAM and the acidity of the solvent, the hydrolysis may be self-limiting since the acidity increases as L-PAM is hydrolyzed. For example, we found that no hydrolysis occurred, even at ≥ 60°C if the acid concentration was approximately 1 N HCl. Other acids were not investigated. In extending these observations, Chang et al (14) found that L-PAM (25 μg/ml in human plasma 37°C) disappeared as a first order process with a rate constant of approximately 0.311 hour^{-1}. Stability of the hydrolysis products in aqueous solution was not formally assessed, but it was observed that at 4°C primarily L-MOH was formed.

Further hydrolysis to L-DOH appeared to require either higher temperatures or a higher pH.

Both hydrolysis products and metabolites appeared to be stable in the sample extracts since duplicate analyses conducted several months apart revealed no change in the profile of radioactivity that eluted from the column. Since the assay procedure was developed primarily for L-PAM, the possibility must be considered that some metabolites may not be stable in the extraction medium. However, when raw urine or bile was injected onto the column and the radioactivity profiles were compared with extracts of the same samples, no apparent quantitative or qualitative differences were observed.

Soon after the initial trials of L-PAM against myeloma, investigators began attempting to improve the therapeutic index and thus provide a greater margin of clinical safety during treatment with the drug. Hundreds of analogs were synthesized (19,22,37). According to Elson et al (19), "Blocking of the free COOH groups of melphalan or its peptides by esterification does not reduce the physiological activity. In contrast, blocking of the free NH_2 group by formylation or acetylation causes considerable reduction in activity, though there is no corresponding decrease in the chemical reaction of the mustard group."

A primary difficulty of the chemical analog approach was the lack of solubility of the compounds under physiologic conditions; they normally required oral administration or injection as a suspension. One such compound, Asaley, was studied in our laboratory. We could not detect parent compound in the blood of mice after oral dosing, although sarcolysin was easily found. No therapeutic advantage over L-PAM was noted.

TOXICITY

Schmidt et al (59) reported on the toxicity of 115 alkylating agents, 20 reference alkylating agents, and 12 nonalkylating agents. The report included extensive observations on the pharmacology and toxicology of L-PAM in mice, rats, dogs, and monkeys. They reported an LD10 for mice and rats of 2–5 mg/kg with considerable variation (as much as 100%) among strains. No sex-related difference in lethality was noted. Subsequent work by Schabel, Griswold, Corbett, and co-workers (unpublished results) at Southern Research Institute suggests that for mice the LD10 may be as high as 10–15 mg/kg. In dogs and monkeys deaths were reported after the iv administration of 0.428 mg/kg of L-PAM daily for 14 days; at one half of that dose (0.212 mg/kg), no deaths were reported. Henry (29) reported that lethality was produced by the same total dose, irrespective of schedule. However, iv dosing was approximately twice as toxic as oral dosing in dogs, a double confirmation of Schmidt's findings since the LD50 values were also in the same range.

Toxicity included anemia, reticulocytopenia, neutropenia and thrombocytopenia, malaise, and diarrhea. In dogs, L-PAM at 5 mg/kg was a powerful emetic, with emesis starting approximately 45 minutes after dosing. Other common findings were atrophy of the spleen, decreased cellularity of the bone marrow, petechial hemorrhage in the skin, and diffuse hemorrhagic lesions in abdominal and thoracic viscera.

In general, the observations by Schmidt hold true for human subjects too. Hoogstraten et al (31,32) reported that leukopenia and thrombocytopenia were seen in most patients given 150 μg/kg/day for 7 days followed by 2 weeks of no therapy. Other symptoms of toxicity were nausea, vomiting, pruritis, and maculopapular rash.

Logue et al (40) found only infrequent drug-related toxicity during L-PAM therapy. Evidence of depressed marrow function was reversible within 2–4 weeks after suspension of therapy. George et al (25) reported that toxicity was minimal when L-PAM (250 μg/kg) was administered daily with prednisone for 4 days with an interval of 6 weeks between treatments. They noted that continuous alkylator therapy was likely to cause granulocytopenia.

With a loading dose of 150 μg/kg/day for 7 days followed by a maintenance schedule of 50 μg/kg/day, bone marrow depression was the most commonly observed and significant form of toxicity, yet it was rarely severe (18). The next most common form of toxicity was infection, probably related to leukopenia.

A more serious consideration for toxicity is the carcinogenic potential of L-PAM. Reports by Kyle et al (35,36) and Ohta et al (50), indicate a possible association of L-PAM therapy with rapidly progressive myelomonocytic leukemia. Reimer et al (57) reported that patients treated with alkylating agents for advanced ovarian carcinoma had a relative risk of 171 for development of acute nonlymphocytic leukemia.

The danger is appropriately emphasized. It is clear that risks versus benefits must be very carefully considered in deciding whether or not to initiate therapy with L-PAM or other alkylating agents.

BIOCHEMICAL AND PHYSIOLOGIC EFFECTS

Timoshechkina and Budilina (62) suggested that sarcolysin caused less blood toxicity when administered in a 10% emulsion of brain tissue. Further-

more, the antitumor activity was not depressed. It is now known that lipid emulsions called liposomes are excellent carriers for certain drugs. A study of pinocytosis in normal versus tumor cells might help to explain this observation.

Bassleer (5) reported that in the presence of mitomycin C or L-PAM, premitotic synthesis of nuclear proteins, nucleolar material, and DNA takes place. Fibroblasts grown in the presence of L-PAM become octaploid and in some cases even hexadecaploid relative to DNA and nuclear proteins. Bassleer and Desaive (6) suggested that sarcolysin inhibited cell multiplication by blocking the cell cycle in prophase. They indicated also that the effects of sarcolysin on Ehrlich cells were the same as those produced on normal fibroblasts.

Cooper and Topping (17) reported the effects of L-PAM on sensitive and resistant strains of Yoshida ascites tumors. They found that the sensitive strain showed a marked fall in mitotic index, an accumulation of cells in G_2, and finally cell death occurring 24–72 hours after treatment. The resistant line showed only slight mitotic inhibition and a possible recovery phase 24–30 hours after treatment with no evidence of cellular damage. Panosyan (51) attributed these results to differences in the histone binding of L-PAM between the sensitive and resistant tumor strains.

Papadakis et al (52) found that L-PAM plus antibody to L-PAM was more effective than L-PAM alone in prolonging the survival of mice bearing intracerebral ependymoblastomas. The observation is interesting from a clinical standpoint since there are essentially two modes of therapy. The first mode requires relatively high doses of L-PAM for short periods of time, conditions under which antibody to L-PAM might be formed but which would be therapeutically ineffective because of the short treatment schedule. The second mode of therapy requires treatment with lower doses of L-PAM over extended periods of time, which could facilitate the development of antibody. The antibody would act essentially as a carrier protein and increase the protein binding of L-PAM. If the protein complex were selectively taken up by tumor cells, it would be easy to understand the apparently high therapeutic activity of low concentrations of L-PAM in the blood. Furthermore, failure to form antibody to L-PAM might reduce the selectivity, but would not necessarily eliminate the sensitivity of the tumor to L-PAM. Indeed, the data from Jeney et al (33) suggest that pretreatment of rats with a low dose of L-PAM 48 hours before toxicity testing nearly doubled the LD50. The protecting effect is apparently not specifically generated by L-PAM since Millar et al (43) reported that pretreatment with cyclophosphamide 7 days before a high dose of L-PAM reduced the damage to intestinal mucosa in sheep. Hedley et al (28) reported that a priming dose of cyclophosphamide 7 days before a high dose of melphalan to L-PAM resulted in a more rapid recovery of peripheral wbcs in comparison with those patients receiving L-PAM alone.

In other experiments aimed at altering the action of L-PAM, Ball and Connors (4) reported that pretreatment with cysteine protected about twofold against L-PAM. They claimed that the protection resulted exclusively from a reduced alkylation of DNA. Unfortunately, they did not indicate whether or not the therapeutic effect was also reversed. Rochlin et al (58) reported a similar study in which the antihistamine tripelennamine was found to be effective in decreasing the mortality associated with L-PAM therapy without altering antitumor effectiveness when administered up to 5 hours after L-PAM in animals bearing Ehrlich ascites tumor. Pashinskii (53) found a similar effect when diuretics such as acetazolamide, chlormerodrin, and hypothiazide were used in combination with L-PAM against Sarcoma 45 tumors in rats. Hickman and Melzack (30) have recently reported that Walker 256 tumor cells are protected against L-PAM toxicity by the imidazoles, and Vistica et al (63) have suggested that the protection could be related to the inhibition of a carrier mechanism since the LD90 for L-PAM against L1210 cells in culture was increased 7.3- and 10.8-fold in the presence of 0.1 mM L-glutamine or L-leucine, respectively. Begleiter et al (7) have recently suggested that there may be two carrier systems with Kms of 80 and 10 μM, respectively. The Km suggested by Vistica et al (64) for a single system was 19 μM. In the same report (64) it was suggested that basic amino acids caused an increased intracellular concentration of L-PAM in cultured cells. The clinical utility of these data is not clear. Although the ability to manipulate a transport mechanism might be useful, if differences in transport exist between normal and neoplastic cells, such differences have not yet been demonstrated. Co-treatment of mice bearing L1210 tumors with L-PAM and an arginine homolog yielded no increased therapeutic effect compared with L-PAM alone (65). Furthermore, the apparent Km of the carrier for L-PAM was approximately 1000-fold greater than the maximum concentration of L-PAM expected in serum after a therapeutic dose of 0.6 mg/kg iv (2).

Using a cell culture of mouse P388 leukemia, Sladek (60) found that centrophenoxine potentiated the toxicity of L-PAM when the cells were exposed to the two agents simultaneously.

Of interest, but of unknown clinical significance,

are the observations of Lesnaya and Lobova (39) who reported that the LD50 for L-PAM in mice varied with the time of dosing. Mortality was maximum when dosing occurred in the morning and late evening. The authors suggest that the maximum mortality periods coincided with high levels of mitotic activity.

Munson et al (46) reported that L-PAM toxicity was not enhanced as a result of pentobarbital treatment. However, this was only a single treatment with the barbiturate and would be expected to have little, if any, effect upon metabolism except perhaps as a blocking agent or competitive inhibitor.

BIOAVAILABILITY

Although L-PAM is most frequently given orally, the clinical literature is lacking in straightforward comparisons of iv versus oral dosing. In the extensive toxicology studies reported by Schmidt et al (59), the oral LD10 for rats was 50%–100% higher than the LD10 from ip dosing. Henry (29) confirmed these observations in dogs. In a clinical study by McIntyre et al (42) standard-risk myeloma patients treated with iv L-PAM actually received less drug but they experienced a higher response rate, a longer duration of survival, and greater myelotoxicity than those treated orally.

In our laboratory ^{14}C-L-PAM given orally to dogs was rapidly absorbed from the gut, and reached a maximum concentration in the serum by 30 minutes. If the area under the serum-concentration in curve is taken as an estimate of bioavailability, then the bioavailability after oral dosing was about 75% for total drug equivalents and about 96% for parent compound (table 1) (24).

In contrast to the very uniform absorption characteristics observed in dogs, Alberts et al (1) reported considerable variability in absorption by human subjects. Based on C × t values, L-PAM was about 72% (range, 30%–100%) bioavailable after oral dosing in man.

Tattersall et al (61) reported that three patients given oral ^{14}C-L-PAM excreted 20%–50% of the radiolabel in their feces over a 7-day period. The fecal excretion was attributed to poor drug absorption from the gastrointestinal tract. Although failure to absorb the drug may have contributed to the high rate of fecal elimination of radioactivity, in our experience with dogs ^{14}C-L-PAM was well-absorbed from the gut, and then rapidly excreted in the bile. Biliary excretion of L-PAM and its products of degradation is also significant in the rat and monkey and will be discussed in a later section of this paper. In our laboratory the dogs were fasted overnight prior to use and they had normal blood chemistry

TABLE 1.—Bioavailability of L-PAM and equivalents in the dog (C × t ng–hr/ml)

	L-PAM	Drug equivalents
Iv	520+	37,000
Orally	500+	28,000
Ratio	0.96	0.77

profiles and renal function, conditions and requirements that are difficult to meet in human studies. We believe that absorption in human subjects can be maximized if the following two procedures are followed.

1. *Treatment should be given on an empty stomach.* This minimizes the number of substances to which the L-PAM molecule might bind or with which an alkylating agent might react. Before-breakfast dosing should meet this requirement.
2. *Oral doses should be given in an acidic solution.* The mechanics of tablet dissolution are complex and unpredictable, and since the therapeutic index is low, the administration of a solution allows more precision dosing to be accomplished. The solution should be acidic to reduce hydrolysis. We have used 0.01 N HCl for that purpose.

DISTRIBUTION

Cohn (16) was first to report on the distribution of radioactivity derived from ^{14}C-sarcolysin. He found radioactivity in liver, kidney, spleen, tumor, thymus, and whole blood, with tumor having the highest activity per gram of tissue. Subcellularly, the radioactivity was found primarily in the protein fraction with kidney protein binding the highest concentration of radioactivity. Novikova (48) reported that after ip injection of ^{14}C-sarcolysin in tumor-bearing rats, radioactivity was detected in tumor, liver, intestine, and spleen within 5–10 minutes. The peak of radioactivity in blood, liver, intestine, and spleen occurred in 15 minutes. Peak radioactivity occurred at 30 minutes in kidney and at 1 hour in tumor. Maximum incorporation in tumor proteins occurred in 2 hours. Nuclei of tissues contained less radioactivity than did cytoplasm. Milner et al (44) reported the distribution of ^{3}H-sarcolysin in kidney, liver, intestine, spleen, tumor, bone marrow, thymus, and muscle of rats receiving the drug ip as a therapeutic dose. They detected very little incorporation of radioactivity in the DNA of sensitive Walker 256 carcinoma, and found no radioactivity in the tumor protein.

TABLE 2.—L-PAM and equivalents in sensitive and resistant tumors*

Time period	L-PAM equivalents in tumor extracts (μg L-PAM-eq/g of tumor)†		L-PAM levels in tumor extracts (μg L-PAM/g of tumor)†		Drug equivalents in precipitates (μg L-PAM-eq/g of tumor)†	
	Resistant tumors	Sensitive tumors	Resistant tumors	Sensitive tumors	Resistant tumors	Sensitive tumors
			L1210			
15 mins	4.65 ± 0.83	16.94 ± 3.84‡	1.03 ± 0.19	3.33 ± 0.61‡	4.08 ± 0.27	4.06 ± 0.36
30 mins	8.08 ± 0.87	7.33 ± 0.61	1.57 ± 0.16	1.50 ± 0.11	4.95 ± 0.47	6.57 ± 0.52‡
1 hr	4.43 ± 0.83	6.74 ± 2.04	0.68 ± 0.14	1.07 ± 0.31	7.79 ± 0.73	10.94 ± 0.87‡
2 hrs	1.58 ± 0.45	3.25 ± 0.85	0.15 ± 0.02	0.33 ± 0.09‡	7.44 ± 0.67	12.93 ± 0.70‡
			P388			
15 mins	5.12 ± 0.29	7.68 ± 0.66‡	1.00 ± 0.06	1.43 ± 0.14	3.28 ± 0.09	4.75 ± 0.19‡
30 mins	4.09 ± 0.35	7.31 ± 0.66‡	0.70 ± 0.04	1.28 ± 0.08‡	4.85 ± 0.29	7.62 ± 0.45‡
1 hr	2.50 ± 0.18	4.66 ± 0.30‡	0.36 ± 0.04	0.73 ± 0.06‡	5.62 ± 0.29	11.08 ± 0.36‡
2 hrs	1.34 ± 0.08	2.50 ± 0.22‡	0.11 ± 0.01	0.25 ± 0.03‡	7.17 ± 0.41	14.68 ± 0.36‡

*Male BDF_1 mice received sc implants of L-PAM-sensitive L1210 or P388 solid tumors on the left side and L-PAM-resistant tumors on the right side. At the time of the experiment the tumors weighed approximately 400–500 mg. ^{14}C-L-PAM was labeled in the side chain. The dosing solution was 1 mg/ml in 11 mM HCl with a specific activity of 6×10^6 picocuries/μg. Purity was 93% for the dosing solution. Each mouse received 10 mg/kg iv, and was killed by exsanguination under ether anesthesia at the appropriate time interval. L-PAM was assayed using HPLC and drug equivalents (total radioactivity expressed as L-PAM) were determined using a Packard-306 sample oxidizer.

†Values = mean ± SE from 5 animals. Values assume uniform distribution of the tumor throughout the extract.

‡$P < 0.05$ that the value for the sensitive tumor is different from that for the resistant tumor.

TABLE 3.—Distribution of total radioactivity in monkeys at 2 different times after iv dosing with ^{14}C-L-PAM at 0.75 mg/kg

Tissue	μg/g*	
	2 hrs†	4 hrs†
Whole blood	0.51	0.47
Serum	0.69(0.22)‡	0.56(0.10)
Liver	1.06(0.21)	0.93(0.08)
Kidney	7.20(2.57)	4.84(0.83)
Spleen	0.54(0.20)	0.39(0.08)
Bile	28.36(2.63)	51.67(3.89)
Heart	0.71	0.65
Lung	0.62	0.59
Pancreas	0.56	0.40
Adrenal	0.47	0.51
Diaphragm	0.55	0.63
Mammary fat	0.21	0.34
Skeletal muscle	0.46	0.41
Bone	0.05	0.07
Bone marrow	0.36	0.29
Urinary bladder	10.66	
Stomach	0.43	0.39
Duodenum	0.59	0.46
Jejunum	1.26	0.72
Ileum	0.62	0.88
Colon	0.48	0.44
Skin	0.25	0.29
Thyroid	0.11	0.17
Ovary	0.43	0.38
Pituitary	0.47	0.47
Submaxillary	0.55	0.39
Parotid	0.42	0.34
Cerebrum	0.22	0.22
Cerebellum	0.23	0.16
Medulla	0.12	0.11

*Total radioactivity expressed as μg of ^{14}C-L-PAM/g of tissue.

†Time monkeys were killed after injection; 1 monkey at each time period.

‡Nos. in parentheses = levels of L-PAM (μg/g) as determined by HPLC analysis.

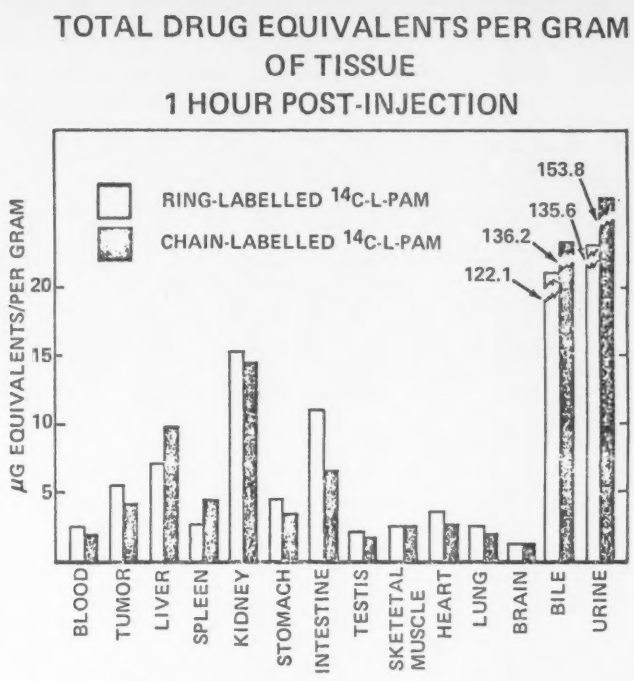


FIGURE 1.—Distribution of ^{14}C-L-PAM in mice bearing Ridgway osteogenic sarcoma after an iv dose of 10 mg/kg, ring or side-chain labeled drug.

In a similar type of study we found that Ridgway osteogenic sarcoma in BDF_1 mice had a level of total radioactivity similar to other tissues, but that a higher percentage of the radioactivity in the tumor was parent compound. It was not clear whether the tumor lacked metabolic capability or whether ^{14}C-L-PAM may have been uniquely stable in the tumor environment similar to the stability of L-PAM in dog bile.

Sensitive tumors of the L1210 and P388 lines contained more radioactivity per gram of tumor than did resistant tumors of the same lines. On the basis of dry weight, total radioactivity in the precipitate of whole tumor homogenate increased with time. Levels were higher in sensitive than in resistant tumors (table 2).

It is likely that a thorough evaluation of the subcellular distribution and metabolism of L-PAM will provide insight to the mechanism of action and the basis for resistance.

With the advent of new technology it became possible to focus upon the parent compound. We found in our laboratory that the highest levels of L-PAM in the mouse were in kidney, liver, skeletal muscle, and heart, respectively, at 1 hour after injection of L-PAM (10 mg/kg iv), while the highest levels of L-PAM in the dog were in bile, urinary bladder, intestine, spleen, liver, and gallbladder at 4 hours after an injection of L-PAM (10 mg/kg iv) (23). Subsequent studies (24) using ^{14}C-L-PAM sustained this observation for the dog. In monkeys dosed with ^{14}C-L-PAM (table 3), a similar pattern of distribution was observed in that the highest levels of radioactivity derived from ^{14}C-L-PAM were found in bile, kidney, duodenum contents, and liver; parent compound comprised 9.3% of the total radioactivity in bile, 36% in kidney, and 20% in liver (11). Similar patterns of distribution have been found for ^{14}C-L-PAM in mice bearing Ridgway osteogenic sarcoma (fig 1) and in Sprague-Dawley rats (table 4). Considering the chemical nature of L-PAM, it is not surprising that distribution in brain was not extensive. However, since the drug is used extensively for suppression of proliferating marrow cells and for breast cancer, it was peculiar that these tissues did not contain higher concentrations of radioactivity.

TABLE 4.—Distribution of total radioactivity in rats at various times after iv dosing with 1.56 mg/kg of ^{14}C-L-PAM

Tissue	μg/g*			
	0.5 hr†	1 hr†	2 hrs†	4 hrs†
Whole blood	2.57 ± 0.07	2.19 ± 0.08	1.52 ± 0.04	0.85 ± 0.09
Serum	4.12 ± 0.29	3.33 ± 0.14	2.15 ± 0.05	0.99 ± 0.12
	(3.26 ± 0.26)‡	(2.58 ± 0.13)	(0.94 ± 0.08)	(0.19 ± 0.03)
Liver	5.18 ± 0.21	4.58 ± 0.39	3.97 ± 0.17	2.16 ± 0.15
	(3.13 ± 0.26)	(2.44 ± 0.26)	(1.24 ± 0.08)	(0.27 ± 0.07)
Kidney	10.79 ± 0.37	9.71 ± 1.12	12.67 ± 0.65	8.79 ± 0.94
	(3.58 ± 0.26)	(2.59 ± 0.26)	(1.51 ± 0.10)	(0.32 ± 0.07)
Spleen	1.53 ± 0.04	1.28 ± 0.04	1.11 ± 0.08	0.71 ± 0.06
	(1.17 ± 0.04)	(0.89 ± 0.03)	(0.55 ± 0.05)	(0.13 ± 0.02)
Heart	2.41 ± 0.09	1.96 ± 0.06	1.30 ± 0.05	0.70 ± 0.07
Lung	2.30 ± 0.05	1.35 ± 0.09	1.36 ± 0.06	0.83 ± 0.10
Pancreas	5.00 ± 0.60	3.31 ± 4.43	3.64 ± 0.32	2.45 ± 0.20
Adrenal	3.26 ± 0.25	2.49 ± 0.12	1.79 ± 0.09	0.75 ± 0.07
Diaphragm	1.95 ± 0.17	1.85 ± 0.05	1.51 ± 0.08	1.11 ± 0.17
Fat	0.42 ± 0.06	0.58 ± 0.09	0.70 ± 0.18	0.51 ± 0.08
Skeletal muscle	1.25 ± 0.05	1.23 ± 0.01	1.03 ± 0.06	0.55 ± 0.04
Bone	0.75 ± 0.06	0.71 ± 0.01	0.67 ± 0.02	0.33 ± 0.04
Urinary bladder	1.40 ± 0.11	1.92 ± 0.63	1.40 ± 0.08	1.26 ± 0.20
Stomach	1.45 ± 0.03	1.40 ± 0.04	1.35 ± 0.18	0.92 ± 0.17
Duodenum	4.18 ± 0.44	4.71 ± 0.72	6.48 ± 1.27	1.96 ± 0.23
Jejunum	5.77 ± 0.81	4.57 ± 0.96	11.51 ± 1.97	7.74 ± 4.52
Ileum	2.13 ± 0.06	2.05 ± 0.35	4.46 ± 1.83	7.92 ± 2.03
Colon	1.61 ± 0.08	1.57 ± 0.02	1.42 ± 0.13	1.80 ± 0.36
Skin	1.78 ± 0.06	1.61 ± 0.11	1.08 ± 0.07	0.64 ± 0.04
Cerebrum	0.29 ± 0.02	0.36 ± 0.00	0.39 ± 0.01	0.25 ± 0.02

*Total radioactivity expressed as μg of ^{14}C-L-PAM/g of tissue.
†Time rats were killed after injection; values are mean ± SE from 4 animals at each time period.
‡Nos. in parentheses = levels of L-PAM (μg/g) as determined by HPLC analysis.

TABLE 5.—Elimination of L-PAM in urine and feces after iv dosing

Species	Urine		Feces
	% of total dose (total radioactivity)	% of dose as L-PAM	% of total dose (total radioactivity)
Dog	44	8	25
Monkey	52	20	8
Rat	25	<4	28 (bile)

ELIMINATION

Urinary excretion of L-PAM has been reported by a number of investigators. Novikova and Chestukhin (47) collected urine from anesthetized rabbits fitted with ureter catheters. They found that approximately 27% of the total radioactivity injected was excreted within 2 hours and that at least some of the radioactivity represented parent compound. Milner et al (44) reported that 25% of the tritrium administered was eliminated in the urine of rats during the first 48 hours. In mouse urine (23) < 2% of the dose injected was recovered as L-PAM within 4 hours, between 4 and 8 hours the rate of elimination of L-PAM was very low, and beyond 8 hours L-PAM was not detectable. Alberts et al (1) reported that 45% of the total radioactivity given to three patients was excreted within 24 hours. Although urinary excretion is an important route of elimination for L-PAM and its products, a significant portion of the dose administered is eliminated in the feces (table 5). It is not known if the elimination is quantitative after biliary excretion, or whether significant portions of the materials excreted in the bile are re-absorbed. In the dog the fecal elimination of drug equivalents is the same after iv or oral dosing (24). Absorption was complete with biliary excretion accounting for the material eliminated in the feces. The data are clear that biliary excretion does occur in all species ex-

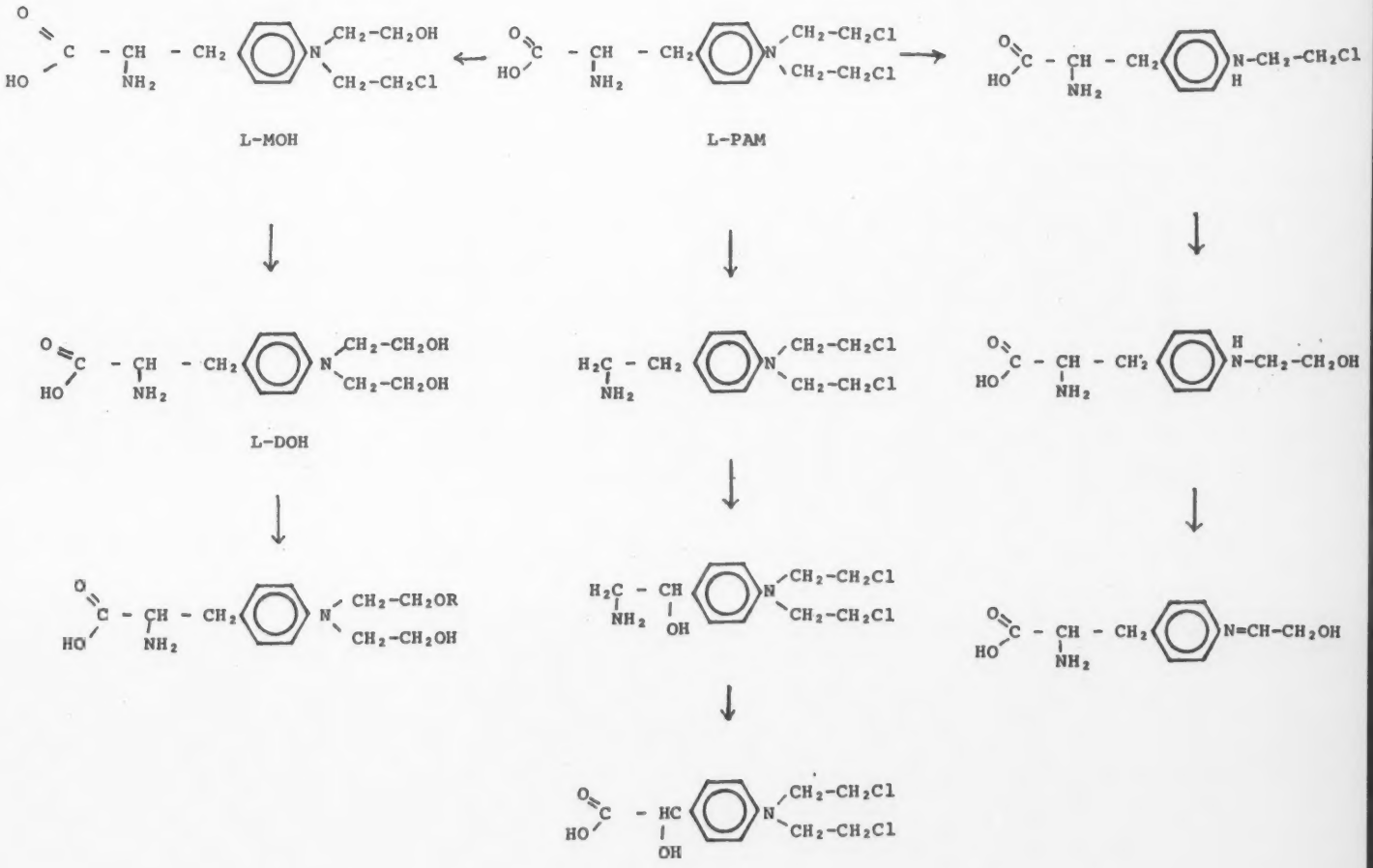


FIGURE 2.—Some possible degradation products of L-PAM.

amined thus far, including the dogfish shark (Furner and Guarino, unpublished results). Additional experiments are required to clarify the mechanism involved and to determine if the route of elimination might be of therapeutic advantage in the treatment of carcinoma of the gallbladder or common bile duct or cancer of the small intestine.

METABOLISM

Although the products of L-PAM, formed by hydrolysis or metabolism, have been studied collectively since 1957, the analytic procedures were not available to permit identification or quantification of individual products.

In 1976 we reported an analytic procedure that made possible the quantification, not only of ^{14}C-L-PAM, but also of a variety of ^{14}C-labeled products formed therefrom. Note that in table 6 there are generally more products detected in the urine and bile than are detected in the serum. A likely explanation is that the products are present in very low concentrations in the serum and are cleared very efficiently.

Mitoma et al (45) suggested that for chlorambucil there could be as many as a dozen metabolites formed from such processes as side chain oxidation, dechloroethylation, esterification, and mustard hydrolysis followed by cyclization. Other pathways which would involve primarily the phenylalanine portion of the molecule are decarboxylation, β-oxidation, and deamination by MAO. Figure 2 illustrates some of those compounds expected. Obviously others could be considered, but hard data are lacking for other than the products of hydrolysis which are L-MOH and L-DOH. These two products are formed by hydrolysis. They have also been prepared in vitro and identified by mass spectrometry after eluting from the HPLC column as described earlier.

The other products have not been identified, although they are apparently formed by similar processes in different species since the chromatographic profiles of extracts of urine, bile, and blood or serum for rat, dog, mouse, and monkey are similar as shown in figures 3 and 4. Benedict et al (8) reported that L-PAM was *activated* in the *Salmonella*-microsome mutagenesis test system, which argues for the formation of at least one active metabolite.

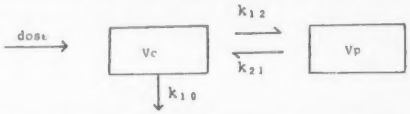
TABLE 6.—Total No. of ^{14}C-labeled products of metabolism or hydrolysis of ^{14}C-L-PAM

Species	Serum	Urine	Bile
Dog	4	4	2
Monkey	1	5	6
Rat	3	5	6
Mouse	3	3	4
Shark	2	7	10
Man	NA*	5	NA*

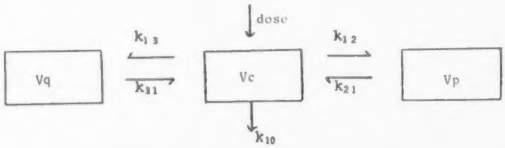
*NA = not available at this time.

PHARMACOKINETICS

Although a basic understanding of the metabolism and disposition of L-PAM may be gained from a study of static systems, it is the dynamic mobility of L-PAM within the body that determines its therapeutic effectiveness. Current knowledge of the pharmacokinetics of L-PAM indicates that the drug is distributed in at least two compartments in the body, eg, a central compartment V_c and a peripheral compartment V_p, as follows:



This is not surprising since a great many compounds are handled by the body in this fashion. However, the disposition of L-PAM within the body need not be limited to two compartments. Indeed a third, or deep, compartment V_q may be easily postulated as follows:



Other models are possible, but at present they offer no obvious advantage. The concentration of L-PAM in the central compartment at any time is a function of the polyexponential equation

$$C = Ae^{-\alpha t} + Be^{-\beta t} + De^{-\delta t}$$

where A, B, and D are 0 time intercepts, α, β, and δ are rate constants, and t is the time at which a given concentration occurs.

Early attempts to assess the pharmacokinetics of L-PAM were filled with frustration. At best the experiments could determine the rate of disappearance of total radioactivity, or total biologic activity, since a specific assay for L-PAM was not available. Nevertheless, the experiments were conducted and the results are in fair agreement with results obtained more recently (table 7). The data of Novikova and Chestukhin (47) appear to be out of phase. However, since their earliest measure-

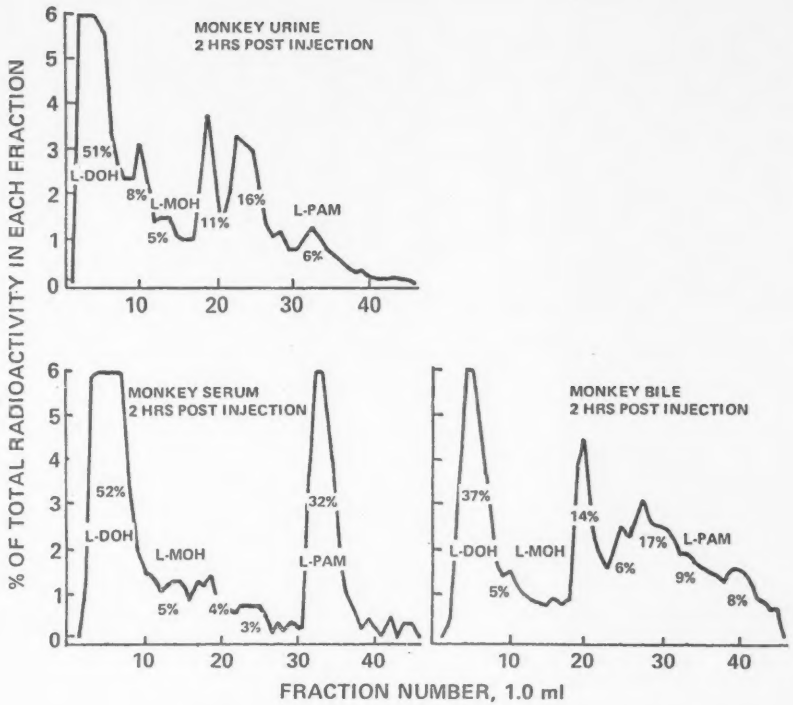


FIGURE 3.—Metabolism of L-PAM in the monkey after 0.75 mg/kg iv.

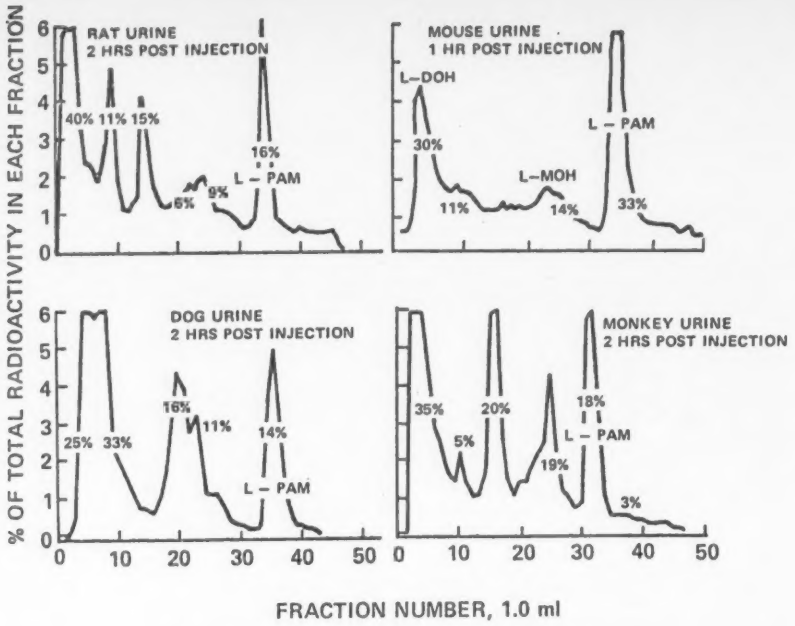


FIGURE 4.—Comparison of urinary excretion of L-PAM and its metabolites.

ment was at 30 seconds, with subsequent samples at 1, 3, 5, 10, 15, and 30 minutes and at 1, 2, and 4 hours, it is possible that they were able to measure a very early phase of distribution in the rabbit that was not observed in any of the other species examined. It is quite clear though that total drug equivalents disappear from serum or whole blood in at least three distinct phases. Although data from the rat suggest only a single phase, this can be explained on the basis of an abbreviated experiment in which the first sample was taken too late to distinguish the rapid distribution phase and the experiment was not continued for sufficient time (due to lack of assay sensitivity) to describe the final or elimination phase.

It is only recently that data on the parent compound have become available. As shown in table 7, all species except the mouse and rat eliminate L-PAM from serum in a biphasic manner. In the mouse and rat, the first sample may have been taken too late to demonstrate a distribution phase, particularly since the data from Novikova and Chestukhin suggest that such a phase in rodents may be quite short. Furthermore, the experiments were not continued long enough to detect a very long half-life.

Although the data demonstrate only two phases of elimination, there is evidence for a third phase since ^{14}C-L-PAM was found in the urine of dogs (24) and more recently in monkeys (11) (fig 5) > 48 hours after dosing. The possibility is that a deep compartment retains some of the L-PAM for a long period of time. Measurement of the L-PAM in serum was not accomplished at those very low levels, but concentration of the drug in the urine at later time periods suggested that it was still being eliminated. The therapeutic implications are exciting since a deep compartment would help explain why low doses are therapeutically effective. If the deep compartment were saturable, overloading could result in toxicity; a better understanding of the characteristics of the deep compartment might help to improve the therapeutic index of L-PAM.

High concentrations of L-PAM in bile were first reported by our laboratory (23). Subsequent experiments confirmed that finding in all species examined thus far. Preliminary studies in the rat demonstrated that the rate of excretion of products of L-PAM into the bile was biphasic with rate constants of 0.33 hour^{-1} and 0.0745 hour $^{-1}$. The rate of excretion of parent compound was monophasic, 0.289 hour^{-1}, through 12 hours (fig 6). The data

TABLE 7.—Pharmacokinetics of L-PAM in serum

	Phases of elimination				
	α	β (hrs)	γ	Species	Investigator (ref No.)
L-PAM	—	0.68	—	Mouse	Furner et al 1976 (23)
	0.24	1.10	—	Dog	Furner et al 1977 (24)
	—	0.87	—	Rat	Furner et al (unpublished results)
	0.10	1.9	—	Monkey	Brown et al 1977 (11)
	0.42	2.8	—	Man	Alberts et al 1978 (1)
Drug equivalents					
	—	3.3	66	Rat	Cohn 1957 (16)
	0.4	6.0	—	Rat	Milner et al 1965 (44)
	< 0.03	≈0.26	> 2	Rabbit	Novikova and Chestukhin 1961 (47)
	0.12	2.8	180	Dog	Furner et al 1977 (24)
	—	1.73	—	Rat	Furner et al (unpublished results)
	0.16	3.8	310	Monkey	Brown et al 1977 (11)

indicate the possibility that a longer phase of elimination may occur but they are not sufficient to statistically demonstrate such a phase. In general, the demonstration of a terminal phase of elimination requires that the concentration of the drug be determined through several half-lives, or to the limits of sensitivity. Thus, little is known of the kinetics of biliary excretion. Clinical observations have reported (12) that L-PAM yielded an objective remission of gallbladder cancer.

Since biliary excretion plays a significant role in the elimination of L-PAM, there exists significant therapeutic potential for the application of L-PAM to treatment of biliary and duodenal cancers. The constraints are that hepatic or biliary dysfunction could compromise the effectiveness of L-PAM used in this manner.

In 25 years L-PAM has proved to be effective against a variety of murine and human tumors, particularly when used as an adjuvant to surgery against human breast cancer. Indeed, Schmidt et al (59) found that, among the alkylating agents, L-PAM was second only to cyclophosphamide in therapeutic effectiveness. Efforts are continuing to im-

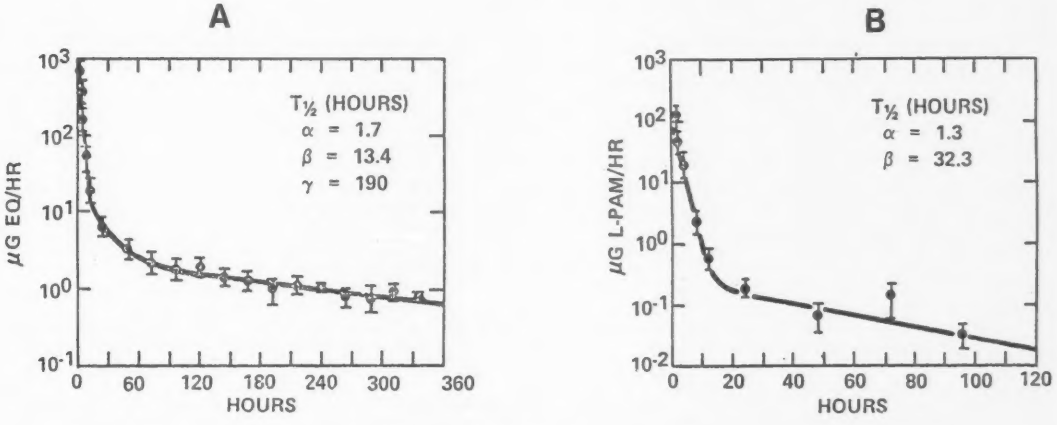


FIGURE 5.—*A*. Urinary excretion of total radioactivity from the rhesus monkey after iv administration of ^{14}C-L-PAM at 0.75 mg/kg. Total radioactivity is expressed as μg of ^{14}C-L-PAM/hr. *B*. Urinary excretion of ^{14}C-L-PAM after iv administration of 0.75 mg/kg. Data = mean ± SE from 4 animals for each time point.

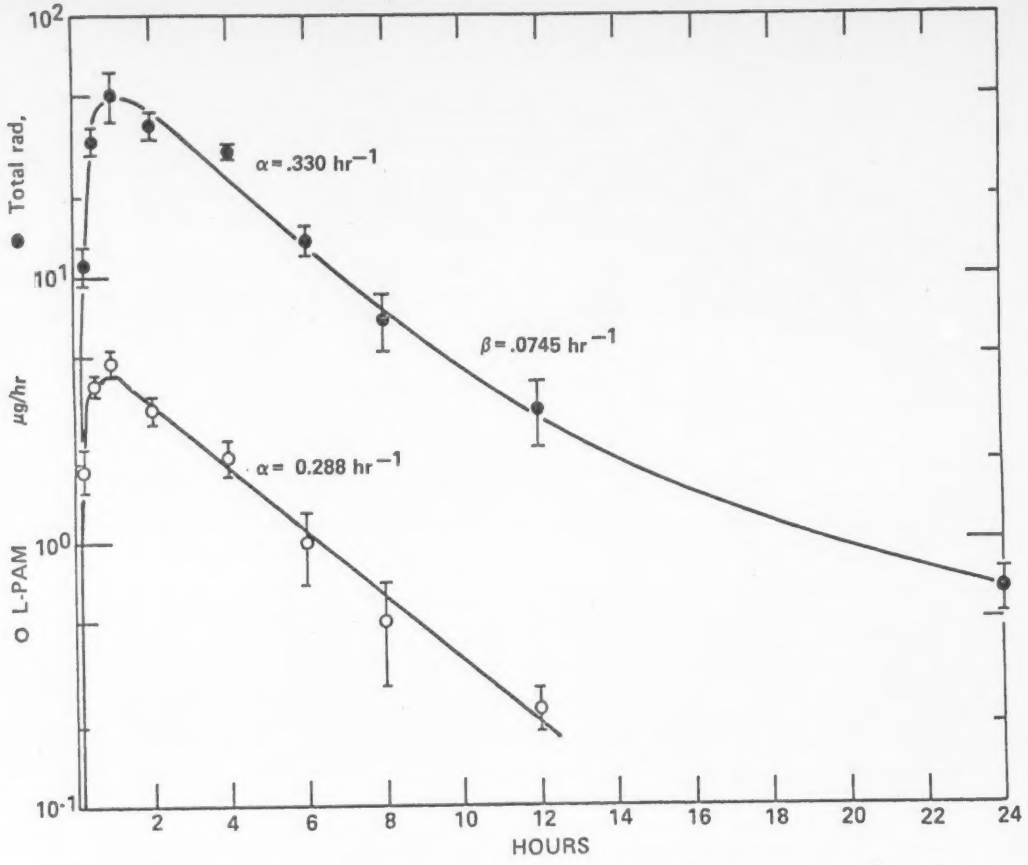


FIGURE 6.—Biliary excretion of ^{14}C-L-PAM and total radioactivity from the rat after iv administration of 1.56 mg/kg. Data = mean ± SE from 4 animals for each time period. ○ = L-PAM (expressed as μg/hr); ● = total radioactivity (expressed as μg of ^{14}C-L-PAM/hr).

prove the rate of therapeutic response by using L-PAM in combination with other therapeutic agents. With the technology currently available it should be possible to define the mechanism and site of action of L-PAM. Only then, can we deliver therapy on a rational basis.

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Distribution, Excretion, and Metabolism of 3-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea (ACNU) in Rats and Mice After Iv Administration[1,2,3]

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SUMMARY

Distribution, excretion, and metabolism of 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea (ACNU) in healthy rats and mice or tumor-bearing mice were studied with the ^{14}C label in each of two positions of the molecule, the ethylene and pyrimidine moieties. After iv injection of 4 mg/kg of ACNU-^{14}C, radioactivity was distributed widely and disappeared rapidly from the body. The half-life of intact ACNU in rat plasma was about 12 minutes. In both animal species, 72%–95% of the radioactivity was excreted into urine within 24 hours. Early dissociation of renal clearance rate was observed between pyrimidine-^{14}C and ethylene-^{14}C, the former being more rapidly excreted than the latter. Ethylene-^{14}C of ACNU was distributed into tumor tissues of X-5563 plasmacytoma-bearing mice, and a tumor level of radioactivity was observed to be twofold higher than the blood level at 3 hours after injection. The concentrations of several metabolites in plasma and urine were determined. Among these compounds, we found an intramolecular carbamoylating product of ACNU. These results suggest that the isocyanate derived from ACNU does not react with any biologic macromolecules, and that the chloroethyl moiety of ACNU has a very important role in the manifestation of the antitumor activity.

[Cancer Treat Rep 64:575–580, 1980]

3-[4-Amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea (ACNU) is an antitumor agent newly synthesized by Nakao et al (1). Because the hydrochloride salt of ACNU is highly water-soluble, the compound can be given iv. Shimizu and Arakawa (2) and Arakawa et al (3) have found that ACNU is active against several experimental tumors and has relatively low toxicity for experimental animals. To clarify the chemical basis of this biologic specificity of ACNU, the metabolic fate of this compound in the body should be studied. The present paper is concerned with the distribution, excretion, and metabolism of ACNU in healthy rats and mice or tumor-bearing mice after iv administration of ACNU labeled with ^{14}C in either the ethylene or pyrimidine moieties.

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[2]This paper constitutes Part II of a series entitled "Studies on Metabolism of ACNU, a New Water-Soluble Antitumor Nitrosourea."

[3]Presented in part at the 10th International Congress of Chemotherapy, Zurich, Switzerland, Sept 18–23, 1977.

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MATERIALS AND METHODS

Animals.—C3H mice and Donryu rats were used throughout this study. Tumor-bearing mice were prepared by sc implantation of plasmacytoma X-

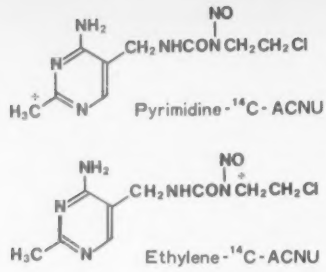

FIGURE 1.—Molecular structure of pyrimidine-^{14}C-labeled ACNU (top) and ethylene-^{14}C-labeled ACNU (bottom). * = labeled position.

5563 (1×10^7 cells/mouse). The tumor-bearing animals received a single iv dose of 4 mg/kg of ACNU-^{14}C 9 days after implantation.

Radioactive materials.—Two ^{14}C-labeled ACNUs were synthesized by Nakao (Central Research Laboratories): ethylene-^{14}C-labeled ACNU (specific activity, 9.1 microcuries/mg) and pyrimidine-^{14}C-labeled ACNU (specific activity, 17.0 microcuries/mg). Positions labeled with ^{14}C in ACNU-molecule are shown in figure 1.

The radioactive purity of these compounds was ascertained to be 97% by thin-layer chromatography (TLC) using two solvent systems, ie, chloroform:methanol (3:1) and *n*-butanol:methanol:water (9:1:1). ACNU-^{14}C hydrochloride was dissolved in physiologic saline just before injection into animals. This compound is stable, at least for several days, in solution kept at 0°C. The dose employed in this study is a minimum effective dose in the BDF_1 mouse L1210 leukemia system (2).

Excretion of radioactivity.—Animals given labeled compounds were housed in a metabolic cage, and urine and feces were collected separately. In some cases, a metabolic cage connected to a β-ray monitor (Triton 955-B, Johnston Laboratories Inc) was used for determining expired $^{14}CO_2$. Portions of urine and fecal suspension were used for determination of radioactivity.

Distribution of radioactivity.—Tumor-bearing mice were killed by bleeding from the carotid artery at various time intervals after dosing. Several organs were weighed and combusted directly with Tri-Carb sample oxidizer (Model 306, Packard Instrument Co) to determine radioactivity. Relatively large or heterogenous organs were homogenized and aliquots of the homogenate were combusted.

Identification and determination of ACNU and its metabolites in plasma and urine.—After administration of ACNU-^{14}C to healthy rats and mice, urine samples were collected in bottles kept on ice for 24 hours. After being lyophilized, radioactive materials were extracted three times with 90% ethanol. By this procedure, up to 90% of the radioactivity was recovered in the ethanol fraction. This fraction was evaporated in vacuo at room temperature to one fifth of the original volume of urine and subjected to two-directional TLC on Kiesel-gel plates (F-254, Merck). Solvent systems used were: chloroform:methanol (3:1, vol/vol) as the first solvent and *n*-butanol:methanol:water (9:1:1, vol/vol) as the second solvent. Development was carried out at 2°C. After drying at room temperature, autoradiograms were prepared with X-ray film (Type-N, Sakura Co. Ltd, Tokyo, Japan). Radioactive spots were identified with co-chromatography with chemically synthesized reference samples. Spot areas of each metabolite were scraped from the plates and transferred to counting vials. Plasma samples were obtained from healthy rats after iv injection of pyrimidine-^{14}C-ACNU. Nine volumes of ethanol were added to 2 ml of plasma and the resulting precipitate was washed three times with the same volume of ethanol at 2°C. Further steps for determination of plasma metabolites were essentially similar to the procedure mentioned above.

Determination of radioactivity.—Radioactivity was determined on a Tri-Carb liquid scintillation spectrometer (Model 3390, Packard Instrument Co). The scintillator solution contained 200 ml of toluene, 800 ml of dioxane, 200 mg of dimethyl-POPOP [2,2′-*p*-phenylene-bis(4-methyl-5-phenyloxazol] and 8 g of PPO (2,5-phenyloxazol). The counting efficiency was corrected by the external standardization.

RESULTS

Excretion of Radioactivity

Excretion of ACNU-^{14}C after iv administration was examined in healthy rats and mice (tables 1 and 2).

In both rats and mice, most of the radioactivity was readily excreted into urine, and excretion into feces was slight. In rats, 4 hours after injection, 32% of the ethylene-^{14}C and 49% of the pyrimidine-^{14}C were recovered from urine. At 11 hours after injection, 93% of the pyrimidine-^{14}C and 69% of the ethylene-^{14}C were excreted into urine. This delayed excretion of ethylene-^{14}C was also observed when mice were given injections of this labeled compound.

Very slight expiration of ^{14}C as carbon dioxide was recognized in both animal species, and it occurred only when ethylene-^{14}C-ACNU was given. It seems, therefore, that the ethylene moiety of ACNU could to some degree be metabolized to one-carbon units in vivo.

TABLE 1.—Excretion of radioactivity in healthy rats after single iv injection of 4 mg/kg of ACNU-^{14}C

Hrs after iv injection	Cumulative % of dose*					
	Ethylene-^{14}C			Pyrimidine-^{14}C		
	Urine	Feces	CO_2	Urine	Feces	CO_2
4	34.1±8.0	ND†	—‡	49.0±3.5	ND	—
11	69.2±7.5	0.2±0.1	2.7±0.5	92.9±1.2	1.2±0.6	ND
24	78.4±6.6	1.4±0.6	—	94.9±1.2	4.1±0.3	—
48	79.5±6.1	2.2±0.5	—	95.4±1.2	4.7±0.3	—
72	79.8±6.0	2.4±0.3	—	95.8±1.2	4.7±0.3	—
Carcass		3.0±0.8			0.4±0.03	
Total recovery		87.9±5.7			100.9±1.1	

*Data expressed as average value ± SE from 3 rats.
†ND = not detected.
‡Dash = not determined.

TABLE 2.—Excretion of radioactivity in healthy mice after single iv injection of 4 mg/kg of ACNU-^{14}C

Hrs after iv injection	Cumulative % of dose*					
	Ethylene-^{14}C			Pyrimidine-^{14}C		
	Urine	Feces	CO_2	Urine	Feces	CO_2
24	71.2	6.3	2.7	81.8	9.0	ND†
48	74.5	7.0	—‡	87.3	9.9	—
72	76.2	7.3	—	91.0	10.1	—
96	76.7	8.6	—	91.3	10.4	—
Carcass		1.5				ND
Total recovery		89.5				101.7

*Data obtained from pooled urine of 3 mice.
†ND = not detected.
‡Dash = not determined.

Distribution of Radioactivity

The radioactivities in blood and various tissues of X-5563 plasmacytoma-bearing mice were determined after administration of ethylene-^{14}C-ACNU (table 3). Radioactivity in the blood rapidly declined within 1 hour after administration. Five minutes after injection, preferential uptake of radioactivity was observed in the liver and kidneys. Levels of radioactivity in the adrenal glands, ovaries, stomach, and salivary glands were slightly higher than those in blood until 30 minutes after dosing. In contrast, transport of radioactivity to tumor tissues was slower than to various healthy organs. About a twofold increase in radioactivity was observed at 3 hours in the blood, and a 1.5-fold increase was observed at 6 hours in the tissues.

A relatively small but noticeable amount of radioactivity was detected in the brain. A similarly small amount was also observed in previous whole-body autoradiographic studies (6). These results might be explained by the moderate lipid solubility of ACNU as a free form (log P = 1.09 at pH 7.4).

Thereafter, the disappearance of radioactivity from various other organs occurred at rates approximately similar to that from blood (table 3).

Urinary Metabolites

Tables 4 and 5 show results of quantitative determination of several metabolites excreted into urine from rats and mice after injection of both ^{14}C compounds. Their chemical structures are shown in figure 2 (14).[6]

[6]Isolation and characterization of these metabolites will be described elsewhere by T. Nishigaki, T. Kinoshita, T. Nakamura, et al.

TABLE 3.—Distribution of radioactivity in tumor-bearing mice after single iv injection of 4 mg/kg of ethylene-^{14}C-ACNU

Time after injection	Concentration (ACNU equivalents, μg/g or ml)*								
	Blood	Tumor	Liver	Kidneys	Stomach	Brain	Salivary glands	Adrenal Glands	Ovaries
5 mins	3.36 ± 0.14	1.20 ± 0.31	10.06 ± 0.44	12.09 ± 2.15	3.17 ± 0.76	1.00 ± 0.04	3.71 ± 0.14	6.30 ± 0.41	5.77 ± 1.89
15 mins	2.64 ± 0.06	1.81 ± 0.33	10.56 ± 0.21	11.17 ± 1.26	3.99 ± 0.37	0.72 ± 0.03	4.71 ± 1.16	6.84 ± 0.74	4.03 ± 0.39
30 mins	2.48 ± 0.01	2.21 ± 0.12	8.99 ± 0.06	8.23 ± 0.31	3.68 ± 0.42	0.58 ± 0	4.17 ± 1.22	5.62 ± 0.58	3.77 ± 0.97
1 hr	0.85 ± 0.15	0.57 ± 0.14	3.56 ± 0.14	4.35 ± 1.38	0.86 ± 0.03	0.22 ± 0.02	1.19 ± 0.42	1.78 ± 0.43	1.33 ± 0.19
3 hrs	0.45 ± 0.05	0.94 ± 0.18	2.01 ± 0.27	1.99 ± 0.21	0.97 ± 0.21	0.17 ± 0.02	0.68 ± 0.17	0.80 ± 0.07	0.86 ± 0.07
6 hrs	0.33 ± 0.04	0.48 ± 0.11	1.02 ± 0.19	0.98 ± 0.20	0.85 ± 0.35	0.09 ± 0.01	0.45 ± 0.14	0.16 ± 0.02	0.42 ± 0.05
24 hrs	0.28 ± 0.03	0.29 ± 0.04	0.75 ± 0.17	0.48 ± 0.02	0.24 ± 0.02	0.08 ± 0.01	0.30 ± 0.04	0.07 ± 0	0.48 ± 0.16

*Each value = mean ± SE from 3 experiments.

Essentially similar results were obtained from urine samples of both animal species; < 0.1% of the dose was excreted as intact ACNU. Rats and mice respectively excreted 23%–29% and 24%–27% of the dose as metabolite D. Metabolites A, B, and C were identified to a lesser extent in both rat and mouse urine. When ethylene-^{14}C-ACNU was injected, about 10% and 19% of the dose (mice and rats, respectively) were located at the origin on the chromatogram. In contrast, radioactivity derived from pyrimidine-^{14}C-ACNU on this area was very low.

Changes in ACNU and Metabolites in Plasma of Rats

Table 6 shows changes in ACNU and its metabolites in plasma of rats after injection of pyrimidine-^{14}C-ACNU. Intact ACNU rapidly disappeared (half-life, 12 minutes) from the plasma. Several metabolites appeared conversely with a decrease in intact ACNU. Significant amounts of metabolite A were produced very rapidly with a peak level at 5 minutes. Concentration of metabolite D increased gradually until 15 minutes. All of the metabolites determined declined thereafter to lower levels.

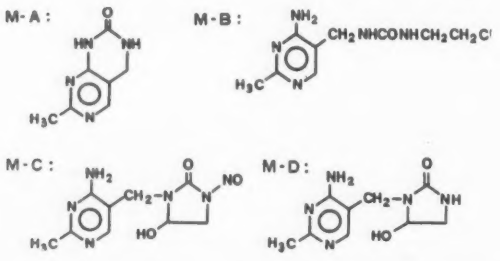

FIGURE 2.—Molecular structure of ACNU metabolites. M = metabolite.

DISCUSSION

Like many antitumor nitrosoureas, such as BCNU, CCNU, methyl-CCNU (4), and chlorozotocin (5), ACNU was shown to have a rather short half-life in plasma (12 minutes in rats, estimated from table 6). It could be, therefore, that this is a common characteristic of nitrosourea compounds. To avoid decomposition of drugs in the process of absorption, iv injection is advantageous for obtaining much higher tissue concentrations of the intact drug within a short time. Rapid distribution of radioactivity following iv injection indicates that a large portion of the intact drug should be effectively transported into various parts of the body including tumor tissues.

In the process of urinary excretion, early dissociation of excretion rates of two labeled ACNUs, ie, pyrimidine-^{14}C and ethylene-^{14}C, was observed, the former being more rapidly excreted than the latter. Shigehara and Tanaka (6) observed similar results in their whole-body autoradiographic study. These results suggest that ACNU is easily degraded to several metabolites, in which pyrimidine-containing entities are more rapidly excreted through the kidneys. On TLC, their R_f values were lower than those for ACNU, indicating that ACNU is converted to more polar metabolites in vivo.

The major transformed product found in the urine was identified as metabolite D. The quantity of this compound in 24-hour urine of mice and rats was almost equal. In vitro experiments have revealed that the step producing this compound and metabolite C depends on hepatic cytochrome P-450-dependent mixed-function oxidase, and this enzyme activity in vitro was of the same order in rats and mice (7). These observations support the finding of metabolite D as the major transformed product both in rats and mice. It is well-known that there is a multiplicity of hepatic mixed-function oxidase activities among various animal species (8,9). In this

TABLE 4.—Urinary metabolites in 24-hr urine of rats after single iv injection of 4 mg/kg of ACNU-^{14}C

Compound	R_f values on TLC*		% of dose†	
	1st direction	2nd direction	Ethylene-^{14}C	Pyrimidine-^{14}C
ACNU	0.79	0.76	< 0.1	< 0.1
Metabolite A	0.56	0.42	—	9.4 ± 1.4
Metabolite B	0.60	0.53	4.6 ± 0.8	7.7 ± 1.1
Metabolite C	0.65	0.51	6.1 ± 1.0	7.7 ± 0.8
Metabolite D	0.39	0.33	25.6 ± 0.9	28.5 ± 0.7
Origin area	0	0	19.3 ± 0.7	3.5 ± 0.8

*1st solvent: chloroform:methanol (3:1, vol/vol); 2nd solvent: *n*-butanol:methanol:water (9:1:1, vol/vol).

†Data expressed as average value ± SE from 3 rats.

TABLE 5.—Urinary metabolites in 24-hr urine of mice after single iv injection of 4 mg/kg of ACNU-^{14}C

Compound	% of dose*	
	Ethylene-^{14}C	Pyrimidine-^{14}C
ACNU	< 0.1	< 0.1
Metabolite A	—	11.1
Metabolite B	7.0	7.1
Metabolite C	3.1	5.1
Metabolite D	23.8	26.8
Origin area	9.5	2.5

*Data obtained from pooled urine of 3 mice.

respect, we are now planning to study the species differences in biotransforming capacity of ACNU in vivo, with special reference to dog and monkey.

Among the identified metabolites, the first metabolite to appear in the plasma of rats was metabolite A. The mechanism of formation of this pyrimidopyrimidine compound is very interesting in relation to the start of biologic activity of ACNU in vivo, because ACNU is hydrolyzed nonenzymatically in aqueous media to produce this metabolite with simultaneous liberation of an active chloroethyl moiety. Furthermore, the chemical structure of this compound makes it easy to consider that an isocyanate, which may be formed during hydrolysis of the ACNU molecule, can immediately carbamoylate the amino group of this isocyanate intramolecularly. This chemical process must be too fast to allow interaction of this isocyanate with other molecules including biologic macromolecules. On the other hand, it is well-established that CCNU exhibits a high carbamoylating activity in vitro and in vivo due to the formation of cyclohexylisocyanate during spontaneous hydrolysis (10–12). This reacts with cyclohexylamine, which is also a degradative product originating from the isocyanate, to form dicyclohexylurea in vivo (13). Additionally, in mouse urine, the occurrence of these metabolites has been described by Oliverio et al (13). However, we could not identify the corresponding dialkylurea-type compound after administration of ACNU. These results suggest that ACNU possesses little carbamoylating potency inherently.

Since no antitumor activities were associated with these metabolites (14), it seems that the antitumor activity of ACNU might be mainly induced by the alkylating moiety. In fact, our results (7) and a recent report by Nagourney et al (15) indicate

TABLE 6.—Plasma level of ACNU and its metabolites in rats after single iv injection of 4 mg/kg of pyrimidine-^{14}C ACNU

Time after injection (mins)	ACNU	Metabolites (nmol/ml)*			
		A	B	C	D
5	3.4	3.9	1.4	1.7	2.1
15	1.9	3.2	1.4	1.7	8.1
30	0.8	2.2	1.4	0.9	4.2
60	0.2	0.5	0.6	0.2	1.1
180	0.1	0.3	0.1	0.1	0.3
360	ND†	0.3	0.1	0.1	ND

*Data obtained from pooled plasma of 3 rats.

†ND = not detected.

that ACNU does not demonstrate carbamoylating activity, although it manifests very high antitumor activity.

The significance of carbamoylation in biologic action induced by nitrosourea compounds is not yet fully clarified. It would thus be worthwhile to investigate a correlation between both the carcinostatic and toxic effects of ACNU and its lack of carbamoylating activity.

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Increased Urinary β2-Microglobulin After Cancer Chemotherapy[1,2]

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SUMMARY

The serum and urinary concentrations of β2-microglobulin (β2-m) and creatinine and the urinary concentration of albumin and IgG were measured in 14 patients with hematologic malignancies before and during chemotherapy. There was a transient increase in urinary β2-m in nine of the 14 patients during chemotherapy. All of the nine patients but only one of the other five patients had had recent multiple chemotherapy. The urinary β2-m increased in 24 courses of chemotherapy given to these nine patients and was already abnormal before 13 of these courses. The increase in urinary β2-m was not associated with an increase in serum β2-m or a significant increase in urinary albumin or IgG excretion. These results suggest that chemotherapy causes a marked transient tubular proteinuria, particularly in patients who have had previous chemotherapy.

[Cancer Treat Rep 64:581–588, 1980]

The serum concentration of β2-microglobulin (β2-m) is often increased in hematologic malignancies including non-Hodgkin's lymphoma and chronic lymphocytic leukemia and may be used to assess the progress of these diseases (1–3). β2-m is readily filtered through the renal glomeruli but is almost totally re-absorbed in the tubular cells so that in normal persons only 0.1% of the filtered load (340 mg/day) appears in the urine (4). In a preliminary study of the urinary and serum concentrations of β2-m in patients with hematologic malignancies given chemotherapy, we observed increases in urinary β2-m which in some patients were disproportionally large when compared to the changes in serum β2-m. These observations suggested that the chemotherapy might have caused tubular proteinuria in these patients. We have studied the effect of chemotherapy not only on serum and urinary β2-m concentrations but also on the urine concentration of two other proteins (albumin and IgG), in an attempt to clarify the effect of chemotherapy on the kidneys.

MATERIALS AND METHODS

Patients

Three groups of patients were studied: 14 healthy subjects (five males and nine females, 20–60 years old), 14 patients with the nephrotic syndrome (eight males and six females, 21–66 years old), and 14 patients with advanced hematologic malignancies undergoing chemotherapy (ten males and four females, 15–67 years old) (table 1). Three of the patients with malignant disease were studied at home and the other 11 were studied while hospitalized.

Serum and Urine Collection

In the patients with malignant disease, urine samples were collected daily for 2 or 3 days before

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[5]We are grateful to Pharmacia (Uppsala, Sweden) for their aid in supplying materials for this investigation and to Dr. A. M. Davison (Department of Medicine, St. James's University Hospital, Leeds, England) for providing the samples from patients with the nephrotic syndrome. We would also like to thank Professor E. H. Cooper (Unit for Cancer Research, University of Leeds) for his help in preparing this manuscript.

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TABLE 1.—Clinical staging and response to treatment of patients with hematologic malignancies*

Patient No.	Age (yrs)	Sex	Disease†	Stage‡	Previous chemotherapy	Clinical response during observation period
1	25	M	ALL		Yes	No
2	66	M	AML		No	Yes
3	15	M	AML		No	Yes
4	73	M	CLL	IV	Yes	No
5	42	M	CLL	IV	Yes	No
6	66	M	CLL	III	Yes	Yes
7	40	M	CGL		Yes	No
8	50	M	NHL	IV	Yes	Yes
9	67	M	NHL	IV	Yes	No
10	67	F	NHL	IV	Yes	Yes
11	62	F	NHL	IV	No	Yes
12	67	F	NHL	III	Yes	No
13	41	F	HD	IV	No	Yes
14	65	M	HMR		Yes	Yes

*At the time of study none of the CLL patients had paraproteins in their sera or urine. Prior to this study a renal biopsy showed minimal-change nephropathy with significant proteinuria (4.6 g/liter) in patient 14. This proteinuria improved markedly after initial chemotherapy.

†ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; CLL = chronic lymphocytic leukemia; CGL = chronic granulocytic leukemia; HD = Hodgkin's disease; NHL = non-Hodgkin's lymphoma; HMR = histiocytic medullary reticulosis.

‡HD and NHL patients were staged according to the Ann Arbor classification (5). CLL patients were staged according to the Rai classification (6).

and up to 8 days after the start of each period of chemotherapy. In initial studies, untimed morning samples of urine were collected, but in later studies 24-hour urine collections were made. Serum samples were taken on alternate mornings whenever possible.

Serum samples and untimed samples of urine were obtained from the patients with the nephrotic syndrome when they attended a renal disease outpatient clinic. Serum and untimed urine samples were collected from nine healthy subjects and 24-hour urine samples were collected from nine healthy subjects.

The pH of all the urine samples was brought above 6.0 with 10 M NaOH where necessary and the serum and urine samples were stored at –20°C.

Chemical Methods

Creatinine was measured in serum and urine initially by a standard Technicon Autoanalyzer method (Technicon Instrument Corp, NY). Later this was done by a method, still under development, proposed by Pye Unicam Ltd, Cambridge, England, using their AC 30 Automatic Chemistry system.

β2-m was measured in serum and urine using the Phadebas β2-micro Test (Pharmacia Diagnostics, Uppsala, Sweden). The standard deviation observed from day to day in measuring a serum with a mean value of 1.5 mg/liter was 0.20 mg/liter and in measuring a urine sample with a mean value of 0.18 mg/liter was 0.02 mg/liter. Albumin and IgG were measured in urine with the Hyland Laser Nephelometer "PDQ" (Travenol Lab Inc, CA) using the general procedure described by Denton et al (7). Standard human serum from Behring Diagnostics (Hoechst Pharmaceuticals, Hounslow, England) was diluted to give standards of 2.0–100 mg/liter for the albumin method and 2.0–63 mg/liter for the IgG method. Nephelometric-grade antisera to human albumin and IgG (Seward Laboratories, London, England) were diluted 1:99 to give the working antiserum solutions. The assay volume of urine or standard was 25 μl. Incubation times were 60 minutes for albumin and 90 minutes for IgG. The standard deviation of 10 values measured over 70 days for a urine sample with a mean albumin concentration of 11.0 mg/liter was 2.0 mg/liter and for a urine sample with a mean IgG concentration of 32.0 mg/liter was 4.0 mg/liter.

Calculation

The rate of excretion of β2-m in urine per liter of glomerular filtrate (GF) was calculated as

$$\frac{\beta2\text{-m}}{GFR} = \frac{[\beta2\text{-m}]u \times V \times [Cr]s}{[Cr]u \times V} = \frac{[\beta2\text{-m}]u \times [Cr]s}{[Cr]u}$$

where β2-m is the amount of β2-m excreted per unit time, GFR is the glomerular filtration rate, [β2-m]u is the urine concentration of β2-m; [Cr]u is the urine

concentration of creatinine, [Cr]s is the serum concentration of creatinine, and V is the urine flow. This index of β2-m excretion does not require a timed urine.

RESULTS

Table 2 shows the effect of 32 courses of chemotherapy involving 15 different drug combinations on the urine concentration of β2-m in 14 patients. The pretreatment value of β2-m for each patient is the value on the day before each treatment course began. The β2-m concentration was considered to be increased by the chemotherapy if the maximum urine concentration of β2-m seen during the 3 days after chemotherapy began was greater than the pretreatment value by at least 2.7 times the standard deviation of the method (0.05 mg/liter). Where more than one value is given for the pretreatment and maximum urinary β2-m concentrations for a patient, the additional values represent the responses to separate courses of the same drug combination. Twenty-four of 32 courses of chemotherapy produced an increase in urinary β2-m in nine of 14 patients treated. All nine patients who showed an increase after treatment had had recent multiple courses of chemotherapy. The urinary β2-m was above the normal range before 13 of these courses and remained above normal after 17 of the courses. Only one (patient 9) of the 5 patients who showed no increase in urinary β2-m after drug administration had had previous chemotherapy, and all had had pretreatment urine β2-m values within the normal range.

Figure 1 shows in more detail the changes in urine and serum β2-m and serum creatinine after drug administration in one patient (No. 8). Figure 2 shows the serum concentration and rate of excretion of β2-m per unit of GF for the 24 courses of treatment which caused an increase in urinary β2-m per liter of GF. Shown are values ($\times 10^{-3}$ mg/liter) before treatment (median, 5.2; range, 0.5–122.2), at peak urinary β2-m (median, 68.1; range, 2.7–609.9), and at the minimum value observed up to 7 days after the treatment day (median, 7.8; range, 0.2–122.2).

The serum β2-m concentration was above the normal range in 18 of 21 pretreatment values but it had not increased at the time of the maximum urinary β2-m per liter of GF. Shown are values (mg/liter) before treatment (median, 3.3; range, 1.3–8.3), at peak urinary β2-m per liter of GF (median, 2.7; range, 1.3–8.1), and up to 7 days after treatment (median, 3.2; range, 1.3–8.2).

Two patients (Nos. 7 and 14) evaluated by 24-hour urine samples and one patient (No. 12) evaluated by untimed urine samples had values for urinary albumin and IgG within the normal range after the first treatment in this study, but these values increased after subsequent treatments and remained elevated for at least 10 days in No. 14 and 2 months in No. 12. The peak values attained in patients 7 and 14 were 63.0 and 638.0 mg/24 hours for albumin and 7.0 and 181.0 mg/24 hours for IgG, respectively. In patient 12 the peak values found were 200 mg/liter for albumin and 100 mg/liter for IgG. Data from these patients were excluded from tables 3 and 4, which show the urinary concentration of β2-m, albumin, and IgG in milligrams per liter and per 24 hours for the other treatment courses in which the urinary β2-m per liter of GF increased above the pretreatment value.

Table 5 shows the ratio of urinary albumin concentration to β2-m concentration at the maximum urinary β2-m per liter of GF in relation to the 24 treatment courses which produced an increase in this index. Values are compared to those seen in healthy subjects, patients with the nephrotic syndrome, and patients with tubular proteinuria.

DISCUSSION

Twenty-four courses of chemotherapy given to nine of the 14 patients in the present study produced transient increases in urinary β2-m excretion without significant changes in the serum β2-m and serum creatinine concentrations. The median urinary β2-m value in milligrams per liter or per 24 hours after chemotherapy was 30 and 83 times respectively the upper limit observed in healthy subjects.

The increase in urinary β2-m excretion after chemotherapy did not appear to be due to changes in the filtered load of β2-m, since the serum β2-m concentration did not increase after chemotherapy. The concentration of urinary β2-m was expressed per liter of GF to exclude changes in the GF as a cause of the increased urinary β2-m. Increases in urinary β2-m occurred whether or not patients responded to chemotherapy; this suggests that the increases were not a consequence of tumor cell destruction.

With untimed urine samples the increase in urinary β2-m concentration was not associated with an increased urinary concentration of albumin or IgG in 11 of 12 treatment courses. The increase in the excretion of β2-m using 24-hour urine samples was associated with an increased urinary albumin and IgG excretion in all of 12 treatment courses. However, the median values for these increases were

TABLE 2.—Effect of different drug regimens on the urinary level of β2-m in patients with hematologic malignancies

Drug regimen	Dose, route, and Days	Patient No.	Pretreatment level of β2-m (mg/liter)	Maximum levels of β2-m after treatment (mg/liter)
Prednisolone	500 mg/m² iv infusion	4	0.50, 0.06, 0.38	6.6, 13.5, 20.9
	500 mg/m² iv infusion	5*	0.11	0.15
	25 mg/m² orally, daily	8	3.7	4.4
Vincristine	1.4 mg/m² iv, Days 1 and 8	7	0.25, 0.09	0.4, 0.8
+				
Prednisolone	40 mg/m² orally, daily			
Chlorambucil	1.5 mg/kg orally, Day 1	6	3.30	13.9
		5	0.09	0.3
Chlorambucil	8 mg/m² orally, daily	10	0.10	0.4
+				
Prednisolone	40 mg/m² orally, daily			
Cyclophosphamide	750 mg/m² iv, Day 1	8	0.30, 0.7	3.8, 4.9
+				
Vincristine	1.4 mg/m² iv, Day 1			
Cyclophosphamide	750 mg/m² iv, Day 1	8	0.67, 0.7	2.5, 5.7
+				
Vincristine	1.4 mg/m² iv, Days 1 and 8			
+				
Bleomycin	4 mg/m² iv, Days 1 and 8			
Cyclophosphamide	750 mg/m² iv, Days 1 and 8	14	6.5, 1.3	26.2, 15.2
+				
Vincristine	1.4 mg/m² iv, Days 1 and 8			
+				
Bleomycin	4 mg/m² iv, Days 1 and 8			
+				
Doxorubicin	20 mg/m² iv, Days 1 and 8			
CVP	Cyclophosphamide, 600 mg/m² iv, Days 1 and 8; vincristine, 1.4 mg/m² iv, Days 1 and 8; prednisolone, 25 mg/m² orally, Days 1–8	12	2.0, 0.9	5.1, 3.7
CHOP	Cyclophosphamide, 750 mg/m² iv, Days 1 and 8; doxorubicin, 50 mg/m² iv, Days 1 and 8; vincristine, 1.4 mg/m² iv, Days 1 and 8; prednisolone, 25 mg/m² orally, Days 1–8	9*	0.06	0.10
		11*	0.09	0.13
BACOP	Bleomycin, 4 mg/m² iv, Days 1 and 8; doxorubicin, 25 mg/m² iv, Days 1 and 8; cyclophosphamide, 750 mg/m² iv, Days 1 and 8; vincristine, 1.4 mg/m² iv, Days 1 and 8; prednisolone, 50 mg/m² orally, Days 1–8	8	1.3	16.1
		10*	0.7	0.7
MOPP	Mechlorethamine, 6 mg/m² iv, Days 1 and 8; vincristine, 1.4 mg/m² iv, Days 1 and 8; procarbazine, 100 mg/m² orally, Days 1–10; prednisolone, 25 mg/m² orally, Days 1–14	13*	0.05	0.06
			0.05	0.06
DAT	Daunorubicin, 50 mg/m² iv, Day 1; cytosine arabinoside, 100 mg/m² iv/sc, 12 hourly, Days 1–5; 6-thioguanine, 100 mg/m² orally, twice daily, Days 1–5	2*	0.06	0.08
		3*	0.13	0.16
Methotrexate	1 g/m² iv infusion	8	0.09, 4.7	6.9, 38.6
with				
Folinic acid rescue	750 mg/m² iv infusion	7	0.09	9.3
	500 mg/m² iv infusion	1	0.10	0.2
Methotrexate	750 mg/m² iv infusion	14	3.4	4.9
Cytosine arabinoside	70 mg/m² sc, Day 1			
Bleomycin	7 mg/m² iv, Day 1			
Mechlorethamine	20 mg/m² intrapleurally	8	0.14	1.8

*Increase in β2-m level not significant.

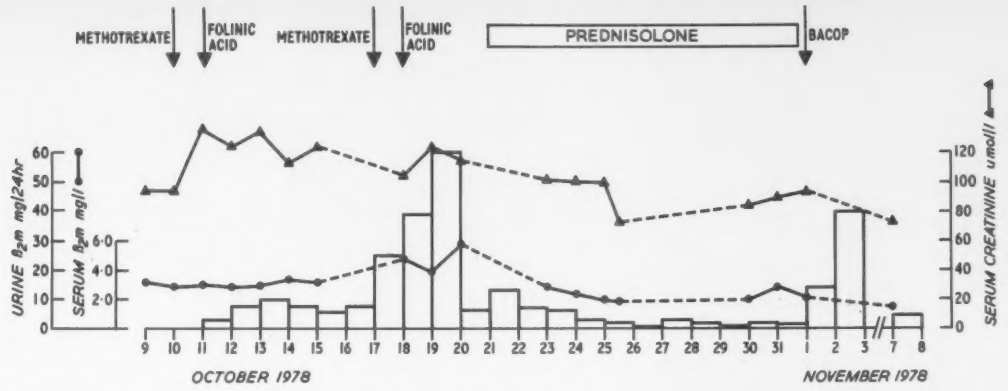

FIGURE 1.—Patient 8 (50 yrs old) with stage IV non-Hodgkin's lymphoma (diffuse mixed histiocytic lymphocytic lymphoma) (*see* table 1). The urinary β2-m increases after each course of treatment (*see* table 2). There is an overall fall in the serum β2-m concentration with progressive treatment. The serum creatinine rises after each methotrexate administration but always remains within the normal range.

within the normal range for healthy subjects in the case of albumin or 1.5 times the upper limit of the normal range in the case of IgG.

The ratio of urinary albumin concentration to urinary β2-m concentration at the maximum urinary β2-m per liter of GF most closely resembles the ratio found in tubular proteinuria. The urinary β2-m concentration was above normal before 13 of the 24 treatment courses and was persistently raised after 17 of these courses. All of these findings together with evidence from other sources (8–10) suggest that the effect of chemotherapy on the renal handling of protein was confined to the tubules and can be described as a tubular proteinuria. Preliminary studies indicate that there is an increased excretion of tubular proteins other than β2-m and an increased excretion of the enzyme *N*-acetyl-β-glucosaminidase, which is taken as a marker of tubular cell damage, after chemotherapy.

Renal impairment in patients with hematologic malignancies is well-documented (11–14), and renal tubular dysfunction has been reported in both myeloma (15) and Burkitt's lymphoma (16). However, the tubular proteinuria in the patients in this study would seem to be associated with multiple chemotherapy since all nine of the patients who had tubular proteinuria after chemotherapy and persistent abnormal urinary β2-m concentrations had received recent multiple chemotherapy. Four of the other five patients who did not experience tubular proteinuria after chemotherapy had not received previous chemotherapy and had normal pretreatment urinary β2-m concentrations.

Both cyclophosphamide and methotrexate are rapidly excreted by the kidneys (17,18) and can cause transient proteinuria and tubular cell necrosis in man (19–21). Therefore, it is not surprising that combinations of drugs containing these agents should affect the urinary excretion of β2-m. The nature of the renal effects of the administration of the other drugs used in this study is still unclear and requires further investigation. Transient elevations of urinary β2-m have been reported in physiologic as well as pathologic situations including febrile illnesses with peak values up to 0.5 mg/24 hours (22), surgical trauma with peak values up to 1.6 mg/24 hours (23) and following arginine or aminoglycoside administration with peak values up to 37.0 mg/24 hours and above 50 mg/24 hours, respectively (24,25). Increases are also seen after strenuous exercise (26) and acute pancreatitis (27).

In conclusion, although the exact mechanism of increased urinary β2-m excretion after chemotherapy remains to be elucidated, the urinary concentration of β2-m may be of value in monitoring the renal toxicity of chemotherapeutic agents. A similar effect seems to be associated with aminoglycoside therapy (24) and the combination of agents that are potentially toxic to the tubules could have severe additive effects.

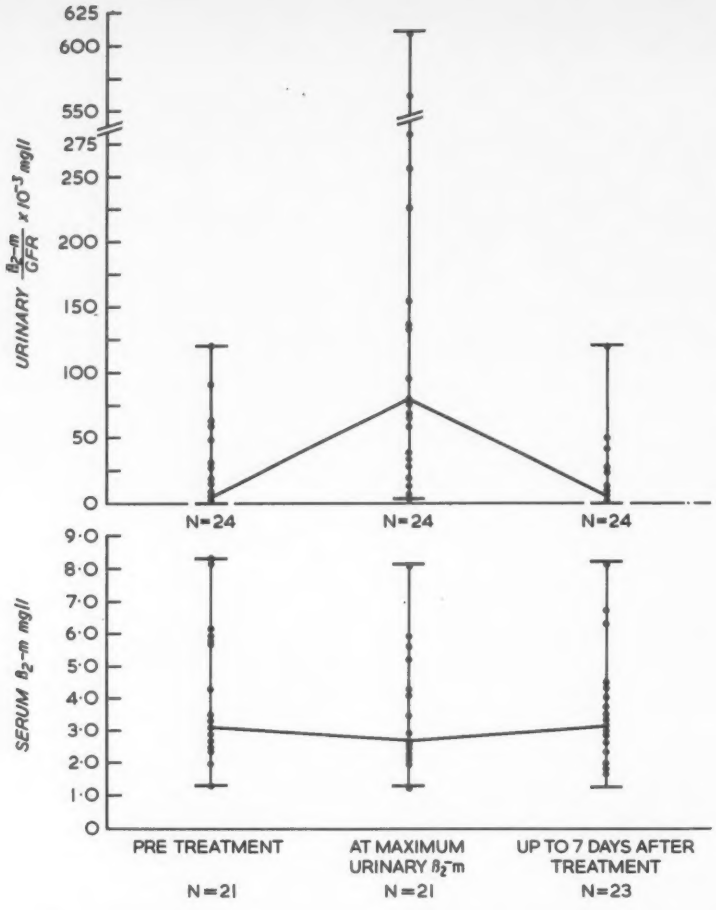

FIGURE 2.—Serum and urine β2-m concentration through 24 courses of chemotherapy in 9 patients with hematologic malignancies. Normal range of values of $\frac{\beta2\text{-m}}{GFR}$ was 0.03–2.3 × 10^{-3} mg/liter, with a median value of 0.7 × 10^{-3} mg/liter. Normal range of serum β2-m concentration is 0.8–2.4 mg/liter but increases with age (ref 4).

TABLE 3.—Urinary concentration of β2-m, albumin, and IgG in 9 healthy subjects and in 5 patients (Nos. 4, 5, 8, 10, and 12) who received 11 courses of chemotherapy: untimed urine samples

Protein	Protein concentration* in—			
	Healthy subjects	Patients before treatment	Patients at peak β2-m value	Patients after treatment
β2-m	0.06 (0–0.16)	0.38 (0.06–2.00)	4.90 (0.34–20.90)	0.38 (0.04–1.30)
Albumin	21 (4–28)	15 (7–33)	14 (1–41)	14 (2–41)
IgG	3 (0–8)	5 (0–18)	4 (0–17)	2 (0–7)

*Median (range) in mg/liter.

TABLE 4.—Urinary values of β2-m, albumin, and IgG in 9 healthy subjects and in 5 patients (Nos. 1, 6, 7, 8, and 14) who received 9 courses of chemotherapy: 24-hr urine samples

Urinary protein	Protein concentration* in—			
	Healthy subjects	Patients before treatment	Patients at peak β2-m value	Patients after treatment
β2-m	0.06 (0.04–0.16)	2.20 (0.09–6.80)	13.3 (0.50–60.20)	2.3 (0.19–6.00)
Albumin	8 (5–32)	11 (3–20)	25 (13–55)	13 (7–30)
IgG	0 (0–4)	1 (0–22)	6 (0–33)	3 (0–11)

*Median (range) in mg/24 hrs.

TABLE 5.—Ranges of the urinary albumin/β2-m ratio in healthy subjects and in patients with nephrotic syndrome, known tubular disorders, and chemotherapy

	Healthy subjects	Patients with—			
		Nephrosis	Tubular proteinuria*	Chemotherapy	
				Untimed urine samples	24-hr urine samples
No.	14	14	15	12	12
Median ratio	187	2464	2.6	4.0	1.8
Range	35–1500	52–173,333	1.0–13.3	0.2–29.4	0.3–72.0

*Data obtained from the literature (ref 8).

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Longitudinal Studies of Cellular Immunity of Patients With Osteogenic Sarcoma During Chemoimmunotherapy[1]

Henry F. Sears,* Richard Simon, and Steven A. Rosenberg[2]

SUMMARY

The cellular immune reactivity of 24 patients with osteogenic sarcoma was studied longitudinally during surgical treatment and subsequent adjuvant chemoimmunotherapy. Cellular immune function was studied using in vitro assays of lymphocyte reactivity to mitogens and nonspecific allogenic antigens. Circulating cells were characterized by the total lymphocyte and wbc counts, determination of E-rosetting populations, and percent of polymorphonuclear leukocytes. Both fresh and cryopreserved lymphocytes were utilized and compared. The preoperative values from these studies offered no useful indication of those patients likely to have disease recurrence. Likewise, longitudinal studies during the course of adjuvant treatment did not predict relapse before other routine clinical studies could identify it. There was no difference in the studied lymphocyte functions and hematologic tests between the group receiving BCG immunotherapy and those not receiving BCG. Our studies indicate that current measures of cellular immune function are of little value in the monitoring of patients with osteogenic sarcoma.

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The cellular immune status of patients with cancer has been studied as a possible indicator of prognosis or response to therapy. Numerous approaches to the evaluation of cellular immune function in patients have been devised including delayed hypersensitivity skin testing and a variety of in vitro assays of cellular immunity (1–12). Patients with osteogenic sarcoma have been reported to have depressed lymphocyte reactivity to mitogens when compared to age-matched controls and patients with soft tissue sarcoma (13). These in vitro tests have yielded conflicting results that could be due, in part, to biologic variables introduced by the tissue culture techniques involved in these assays. The ability to cryopreserve and subsequently recover and test circulating lymphoid cells offers the potential opportunity to overcome some variables inherent in these in vitro assays.

We have previously shown that cryopreserved lymphocytes can be successfully used in blastogenic assays, and that simultaneous assays can reduce the variability of results (14,15). We have collected lymphocytes and sera from a group of patients with osteogenic sarcoma, before and during their treatment course, and have cryopreserved these for storage. Blastogenesis and E-rosette formation were studied longitudinally on both fresh and cryopreserved material from patients undergoing a protocol-directed multimodality course of therapy. The treatment included excision of the primary tumor by amputation and chemotherapy with methotrexate. One half of the patients were randomly assigned to BCG immunotherapy. The results of these longitudinal assays and the hematologic findings from these patients form the basis of this report.

MATERIALS AND METHODS

Clinical Aspects

Studies on the first 24 patients entered in a ran-

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domized protocol for the treatment of osteogenic sarcoma are presented in this report. Each patient received a complete clinical evaluation prior to randomization and stratification, which eliminated patients with clinical evidence of systemic spread from this group. Patients then underwent amputation and postoperatively were started on an 18-month course of high-dose methotrexate and citrovorum factor rescue. Approximately one half of the patients were randomized to receive BCG by Heaf gun intradermal injection. Two patients who were randomized to receive the BCG subsequently refused BCG treatments and did not receive this agent. The results of assays done on these two patients were included in the non-BCG group.

Approximately one half of each group of patients have had a clinical recurrence of their tumor. These were noted either on a chest roentgenogram obtained every 3 weeks or on whole-lung tomograms obtained every 3 months. All recurrences were histologically documented.

Blood was obtained for study preoperatively, postoperatively, and prior to each 3-week course of chemotherapy. Blood was drawn from a number of patients, particularly those receiving BCG, at intervals during the 3–week cycle of chemoimmunotherapy. Serum and lymphocytes were separated and aliquots were used in studies performed on fresh material. A cbc was done and the lymphocytes were cultured with phytohemagglutinin (PHA) and prepared for E-rosette determinations. The remainder of the lymphocytes were cryopreserved for use in simultaneous assays at a later date.

Cryopreservation

Ficoll/Hypaque-separated lymphocytes were washed three times and were suspended in RPMI-1640 medium supplemented with penicillin, streptomycin, glutamine, Hepes buffer, and 10% pooled human AB serum and 10% dimethylsulfoxide. One-ml aliquots were cooled at a rate of 1°C/minute to –50°C in a Planar freezer as previously described (14) and then stored in the gas phase of a liquid nitrogen freezer.

At the time of assay, the lymphocytes from each collection day from one patient were removed from the freezer, rapidly thawed in a 40°C water bath, washed, and used in the appropriate assays. Each test included all of the material gathered from the multiple collections from one patient. These were studied together as a group. Serum was eliminated from the recovery washing media for those cells used in E-rosette formation assays.

Assays

Blastogenesis

One hundred thousand lymphocytes suspended in RPMI-1640 medium with penicillin, streptomycin, Hepes buffer, glutamine, and 20% human AB pooled serum were dispensed into the wells of a Falcon 3040 culture plate in 100-µl quantities. Triplicate samples were stimulated with either 2 µg of PHA-P (Difco) or 0.5 µg of purified protein derivative (PPD) (Parke, Davis and Co, Lot 974-562-B) suspended in media. Medium alone was added to the control wells. PHA-stimulated plates were cultured for 72 hours and the PPD plates were cultured for 120 hours. Dose-response curves for PPD had previously identified the optimal stimulating dose. For comparisons of fresh and cryopreserved cell activity, 100,000 dpm was arbitrarily picked as a division between normal and abnormal reactivity. This figure is supported by the large number of normal samples tested as controls for other studies.

Mixed Lymphocyte Culture

A large quantity of normal lymphocytes were obtained from one donor at the Plasmapheresis Program, Blood Bank, National Cancer Institute, National Institutes of Health (Bethesda, MD) after the extraction of platelets for clinical transfusion. These were separated on a Ficoll/Hypaque gradient and cryopreserved as described above. Prior to cryopreservation, one half of these cells were exposed to 2000 R irradiation. These cells had < 1/100th the activity of their untreated counterparts when tested by stimulation with PHA. They were used as stimulators in the one-way mixed lymphocyte culture (MLC) and as a reference control to judge the activity of different groups of assays performed throughout this study.

One hundred thousand lymphocytes in 100-µl quantities were plated either with another aliquot of similar cells or with 100,000 irradiated reference cells. Two hundred thousand irradiated reference cells were also plated in triplicate. The cells were cultured for 120 hours in a humidified 5% CO_2 atmosphere at 37°C. During the last 6 hours of culture, 10 µl of phosphate-buffered saline containing 1 microcurie of tritiated thymidine (Amersham/Searle, [methyl-^{3}H]thymidine, 5.0 curies/mol) was added to each well.

At the end of the culture period the cells were harvested onto filter strips by means of a multiple automatic sample harvester (Microbiological Associates). The filters were allowed to air-dry overnight, were placed in scintillation vials with Econofluor (New England Nuclear Corp) scintillation

fluid, and were counted on a Packard 2875 scintillation counter. Stimulation indices (test sample/control samples) were calculated for the MLC, but the actual dpm of the control sample subtracted from the test sample were used for most comparisons. Normal donors usually have stimulation indices in the 8–12 range in this assay.

E-Rosette Formation

The prepared lymphocytes, either fresh or thawed after cryopreservation, were suspended in RPMI-1640 medium at a concentration of 2×10^6 cells/ml. An aliquot containing 0.25 ml of the cell suspension was added to 0.2 ml of thrice-washed sheep erythrocytes at a 1:10 ratio for 30 minutes at 4°C. Triplicate samples were prepared in 5-ml plastic centrifuge tubes (Falcon 2063), were allowed to stand at room temperature for 15 minutes, were centrifuged at 280 × *g* for 5 minutes, and were then placed in a 4°C refrigerator for 18 hours. Twenty minutes prior to reading, the tubes were warmed to room temperature. The cell pellet was gently resuspended with a Pasteur pipette and two drops of 0.05% toluidine blue solution were added. An aliquot was transferred to a hemocytometer where 200 cells were counted at 640 × magnification. Conventionally, any lymphocyte with three or more adherent sheep erythrocytes was considered an E rosette. Results were expressed as the means of the three samples.

Statistical Methods

The effect of lymphocyte cryopreservation on the results of in vitro assays was evaluated using the blocked Wilcoxon test (16) (blocked by patient). This test was also used to evaluate whether the in vitro assays or hematologic parameters were of value as early predictors of clinical disease recurrence. Cox's life-table regression test (17) was used to evaluate whether pretreatment in vitro assay results or hematologic parameters were useful predictors of disease-free interval. The effect of methotrexate dose and BCG administration on in vitro assay results and hematologic parameters was evaluated using a repeated measure analysis of variance (18). Two-tailed statistical significance tests were used throughout.

RESULTS

The results of longitudinal tests were tabulated and graphed for each patient together with the important clinical events for that patient. Graphs were made of the longitudinal changes of PHA stimulation and percent E-rosette formation based on the results derived from both fresh and cryopreserved lymphocytes. Figure 1 shows a pair of graphs generated by one patient's results using both fresh and cryopreserved lymphocytes. As can be noted, there is not complete agreement in absolute values of these determinations at one point during the treatment course, but the changes over time did coincide for both fresh and cryopreserved cells. Usually, differences were related to technical factors. The use of a reference lymphocyte culture was helpful in identifying tests in which technical factors might have been responsible for unexpected results.

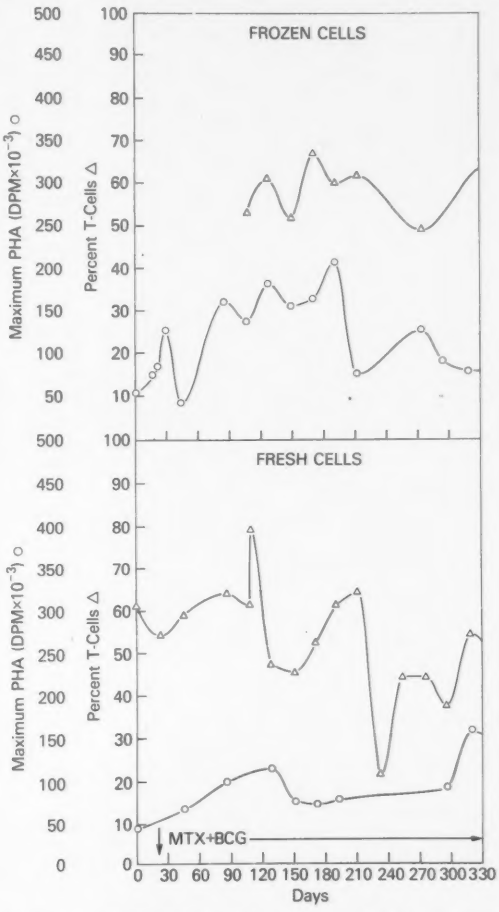

FIGURE 1.—Comparison of fresh and frozen lymphocyte T-cell % and mitogen stimulation. △ = % of E rosettes in the fresh and frozen aliquots of a patient's peripheral blood. ○ = PHA blastogenesis and reflects a profound depression of reactivity. Normal volunteer reactivity in all these assays done simultaneously was 80,000–100,000 dpm.

Table 1 shows the preoperative values for percent E rosettes and PHA stimulation for both fresh and cryopreserved lymphocytes. The preoperative MLC stimulation index, hematologic parameters, and time until disease recurrence are also shown. A slightly higher PHA-induced stimulation was noted in the studies using frozen lymphocytes compared to fresh lymphocytes, but this difference was not statistically significant. T-cell levels of the fresh and frozen material were very similar.

Figure 2 shows the relationship of response of fresh and cryopreserved lymphocytes of all studies that were obtained before the start of chemotherapy. If 100,000 dpm is used as a division between the normal and low PHA response, only one study had significantly lower and two studies had marginally lower values of fresh reactivity compared to that produced by frozen lymphocytes. Conversely, there were three results representing frozen cell function > 100,000 dpm while the stimulation in fresh material was reduced. Thus, four to six of 26 studies could be interpreted differently depending upon whether values for fresh or frozen cells were used. Likewise, the scattergram identifies approximately four instances in which E-rosette percentages could be interpreted one way using fresh cell-generated values and another way using cryopreserved cell values if a value of 60% was used as a point of separation.

Seventeen of a possible 60 determinations using fresh material were lost from the clinical study (table 1) for reasons such as poor recovery, technical error, etc, while 11 of 60 determinations were lost using cryopreserved material. The latter number is higher than would usually occur because of an unexpected move of the stored material to a new laboratory after the study was initiated.

Evaluation of Preoperative Parameters for Predicting Time Until Disease Recurrence

Cox's life-table regression method was used to evaluate whether any of the preoperative in vitro assays or blood cell counts were of value in predicting time until disease recurrence. The parameters examined were % E rosettes, PHA stimulation, MLC, and lymphocyte counts. None of these parameters was found related to time until disease recurrence to a statistically significant degree. The means, medians, standard errors of the means, and

TABLE 1.—Time to recurrence and preoperative values of in vitro assays of cellular immunity and hematologic parameters of patients with osteogenic sarcoma

Patient No.	BCG immunotherapy	% E rosettes		PHA stimulation*		MLC stimulation index	Wbc count ($\times 10^{-3}$/mm^3)	% polymorphonuclear leukocytes	% lymphocytes	Time to recurrence (days)
		Fresh	Frozen	Fresh	Frozen					
1	Yes	47	45	—	62	8	6.1	47	49	300†
2		51	55	143	254	10	8.8	61	35	380†
3		60	60	108	154	3	4.5	48	45	578†
4		71	—	—	141	8	4.7	50	44	620†
5		—	57	—	62	4	6.2	63	32	640†
6		—	—	—	199	—	5.9	67	31	700†
7		78	55	118	162	6	4.6	60	34	158
8		—	48	78	66	3	10.4	69	30	99
9		42	42	137	160	3	8.4	64	28	147
10		62	—	110	135	15	7.3	66	28	268
11		67	—	65	—	—	8.0	74	22	39
12	No	—	63	—	130	8	—	—	—	487†
13		73	70	101	23	4	7.9	60	28	547†
14		66	53	127	371	19	4.5	54	29	700†
15		—	71	102	86	8	5.9	63	37	700†
16		82	60	88	114	8	5.2	65	33	517†
17		59	—	95	263	5	5.1	45	38	98
18		56	36	112	62	5	7.1	50	45	77
19		46	58	—	83	14	4.4	51	38	360
20		41	—	—	12	14	5.1	49	49	156
21		56	66	82	70	16	5.2	66	31	222
22		—	56	—	275	7	6.8	70	25	155
23		44	—	57	—	—	5.4	57	36	423
24		61	63	27	198	26	5.6	51	43	70

*Dpm $\times 10^{-3}$ [methyl-^{3}H]thymidine uptake.
†No recurrence in this many days.

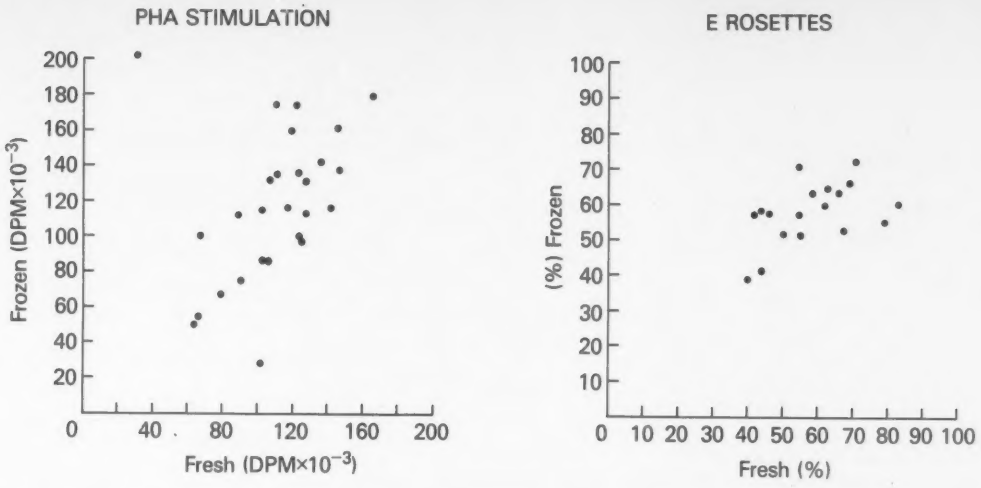

FIGURE 2.—Comparison of fresh vs frozen lymphocytes in preoperative assays. Each point is plotted by location of the value for each fresh and frozen sample of peripheral blood in which the assays were conducted simultaneously.

sample sizes for these parameters are shown in table 2 separately for patients who did and did not have disease recurrence. There was no prognostic value of any pretherapy parameter in these patients.

Effects of BCG and Methotrexate Dose

For each in vitro assay and blood cell count, the mean result for each sample taken 19–21 days after a dose of < 250 mg/m² of methotrexate was calculated for each patient. The mean was also calculated for each sample taken 19–21 days after a dose of 250 mg/m² of methotrexate. Complete data were available for 18 of the 24 patients. A repeated measure analysis of variance was performed for each parameter (seven in vitro assays and blood cell counts) to evaluate the effect of methotrexate dose and BCG on the parameter. For each of the parameters no significant effect of methotrexate dose or BCG or an interaction between these two factors was found (table 3).

If the results are viewed with respect to when during the cycle of chemotherapy and chemoimmunotherapy the blood was obtained, there is an interesting trend (table 4). Again, however, for the group as a whole, there is no statistical difference between the results obtained 1–6 days after methotrexate, those obtained 7–12, 13–18, and 19–22 days after methotrexate, or those obtained just

TABLE 2.—Pretreatment assay values for patients with osteogenic sarcoma

	No recurrence					Recurrence				
		Assay values					Assay values			
Pretreatment assay	No. of patients	Mean	±	SEM	Median	No. of patients	Mean	±	SEM	Median
PHA (dpm × 10^{-3})	10	139.70	±	32.68	105	11	135.09	±	25.84	83
MLC (dpm × 10^{-3})	10	8.0	±	1.42	8.0	11	10.3	±	2.2	6.5
Wbc count (cells/mm³)	10	5970	±	451	5900	13	6377	±	491	5500
% neutrophils	10	57.8	±	2.3	60.0	13	59.4	±	2.6	58.5
% lymphocytes	10	36.3	±	2.3	33.0	13	34.4	±	2.2	32.5
Total lymphocyte count (cells/mm³)	10	2120	±	175	2000	13	2138	±	147	1900
% E rosettes	9	59.8	±	2.8	58.5	8	53.0	±	3.6	55.0

TABLE 3.—Effect of methotrexate dose and BCG immunotherapy on cell-mediated immunity and hematologic values for patients with osteogenic sarcoma*

	Time of assay in relation to surgery and methotrexate dose	Assay values	
		Without immunotherapy	With immunotherapy
% T cell	Preoperatively	56.0 ± 3.0†	58.7 ± 3.6
	< 250 mg/m²	58.7 ± 2.9	56.2 ± 3.3
	250 mg/m²	52.2 ± 6.2	53.7 ± 3.2
PHA stimulation (dpm × 10^{-3})	Preoperatively	141.5 ± 29.8	132.7 ± 19.1
	< 250 mg/m²	160.0 ± 25.5	168.4 ± 16.2
	250 mg/m²	169.2 ± 25.2	175.1 ± 21.8
MLC SI	Preoperatively	11.2 ± 1.8	9.8 ± 3.4
	< 250 mg/m²	8.6 ± 1.6	7.3 ± 2.0
	250 mg/m²	8.7 ± 1.6	7.0 ± 2.1
Wbc count (× 10^{-3})	Preoperatively	5.7 ± 0.3	6.7 ± 0.6
	< 250 mg/m²	5.4 ± 0.5	6.4 ± 0.6
	250 mg/m²	5.3 ± 0.4	6.2 ± 0.5
% polymorphonuclear leukocytes	Preoperatively	56.8 ± 2.3	60.8 ± 2.7
	< 250 mg/m²	48.8 ± 3.8	53.8 ± 2.7
	250 mg/m²	48.3 ± 3.3	49.9 ± 3.6
% lymphocytes	Preoperatively	36.0 ± 2.1	34.4 ± 2.5
	< 250 mg/m²	42.9 ± 3.7	39.3 ± 2.6
	250 mg/m²	42.6 ± 3.9	38.6 ± 4.0

*Values obtained preoperatively and 3 wks after methotrexate treatments.
†Mean and SEM. SE reflects variability among patients.

before the start of the next cycle. For patients not receiving BCG, an apparent depression of lymphocyte activity is seen in the first days after methotrexate which gradually increases for each 6-day period until it is at normal levels immediately prior to the next cycle of drug. This fall and rise of activity is not seen in the patients receiving BCG. They present a very stable pattern throughout the entire 3-week course (figure 3). For this analysis only patients who had values obtained at least once within 19 days after methotrexate are included. Because most of the patients not receiving BCG did not return during the interval between methotrexate administrations, few observations are available for these patients, and conclusive statements cannot be made.

Immunologic Surveillance

Mitogen stimulation and E-rosette percentages for each patient were plotted against time, with the significant clinical events noted. Evaluation of these graphs generated by simultaneous assay of the lymphocytes collected longitudinally during the entire treatment course did not show a consistent recognizable difference between BCG- and non-BCG-treated patients. The same was true when differences were sought between the graphs of those patients who had disease recurrence and those who did not.

For patients who relapsed, the values of in vitro assays and hematologic parameters during the 2 months prior to relapse were compared to corre-

TABLE 4.—PHA stimulation by interval after methotrexate administration

Days after methotrexate dose	Stimulation index*	
	With BCG immunotherapy	Without BCG immunotherapy
1–6	149 ± 40 (6)	63 ± 6 (6)
7–12	172 ± 81 (4)	97 ± 36 (2)
13–18	180 ± 136 (2)	118 ± 35 (2)
19–21	170 ± 44 (7)	145 ± 115 (3)
Total	165 ± 49 (7)	110 ± 61 (3)

*Mean and SDs are calculated on a patient rather than assay basis. Only patients with at least 1 assay < 19 days after methotrexate administration are included. No. of patients is shown in parentheses.

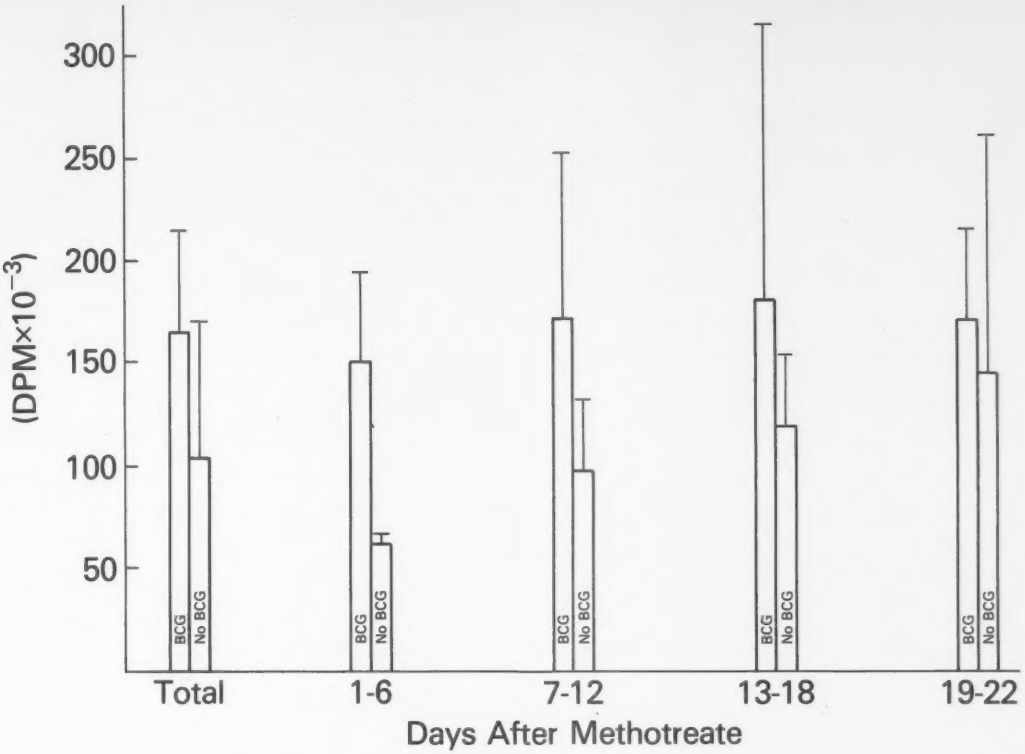

FIGURE 3.—Comparisons of PHA stimulation during the cycle interval of methotrexate treatment and with or without BCG immunotherapy. No. of patients not receiving BCG returning for intercycle assays is small, making significance testing of comparisons impossible (*see* text).

sponding values for the same patient during the preceding 4 months. Only values for samples taken 17–24 days after methotrexate administration were used. Ten patients with disease recurrence had at least one appropriate value in the 2 months prior to recurrence and at least one appropriate value in the preceding 4 months. A blocked Wilcoxon test (blocked by patient) was used to evaluate whether any of the parameters were changing prior to relapse. If such changes were found, it was anticipated that Cox's life-table regression method with time-varying co-variates would be used to evaluate if similar changes occurred in the nonrelapsing patients. The parameters examined were percent E rosettes, PHA stimulation-induced uptake of isotope, MLC stimulation index, wbc count, percent polymorphonuclear leukocytes, percent lymphocytes, and total lymphocytes. No differences approaching statistical significance were found for any of these parameters.

DISCUSSION

In vitro assays of cellular immunity have been extensively studied in patients with cancer. It has been proposed that evaluation of a patient's cellular immune system may provide prognostic information (1–13) and provide a more rational basis for therapy (19). Many of the cellular immunity studies, especially those using in vitro assays, have produced conflicting results (20–27). One of these studies suggested that 80% of patients with osteogenic sarcoma had depressed lymphocyte reactivity to the plant mitogen PHA (13). Coincidentally, this was also the same percentage of patients who could

be expected to relapse with systemic disease after amputation of the primary tumor (28). Because adjuvant chemotherapy appears to have a beneficial role in the management of the patient who has osteogenic sarcoma (29,30), the definition of patients likely to develop systemic spread would be of great clinical value.

Alterations in cellular immunity have been found to occur during some cancer treatments. Longitudinal studies during treatment offer advantages over assays at a single time. Adequate chronologic studies are hampered by numerous variables inherent in the culture techniques employed during in vitro assays.

A well-defined group of patients with osteogenic sarcoma treated at the National Cancer Institute in a multimodality adjuvant protocol formed the basis of the present study (31,32).

These patients underwent serial assays of cellular immune status, including measurement of blastogenesis to mitogens, PPD, and allogenic stimulator cells in a mixed-leukocyte reaction as well as measurement of E rosette-forming cell populations. Results of these studies were used to evaluate if pretreatment responses correlated with or predicted a poor response to therapy or early recurrence of disease. Longitudinal studies were performed to identify any specific changes in cellular immune responses related to BCG immunotherapy.

To minimize biologic variables of these culture assays, both fresh and cryopreserved lymphocytes were used. Our previous studies and those of others (33–36) have shown cryopreserved cells to be functional in these cellular immune assays. A cryopreserved stimulator cell harvested in large quantities from one individual at one time was used throughout the entire study for MLC assays.

None of the in vitro assays of cellular immunity performed on material either collected preoperatively or postoperatively, prior to the start of chemotherapy, correlated with the subsequent clinical course. One half of the patients had disease recurrence during the course of this study, and no unique alteration in blastogenesis, MLC, or E-rosette percent was noted in those who had disease recurrence. Likewise, there were no correlations with the hematologic parameters: wbc counts, percent polymorphonuclear leukocytes, percent lymphocytes, or total lymphocytes.

Neither methotrexate in multiple different doses during clinical escalation of the drug over time nor the use of BCG resulted in consistent, predictable, or statistical changes in the cellular immune status determined by these assays.

The only suggestive difference seen in these patients was noted in the evaluation of the immune status during the cycle of chemotherapy. Those patients not receiving BCG appeared to have a greater depression of their PHA response in the first 6 days following methotrexate than patients receiving BCG. Only a small number of non-BCG-treated patients had observations made during the interval between treatments and therefore the number of observations is small. This trend is worthy of further study.

Using these techniques, comparison of results from one patient or group of patients did not allow early prediction of relapse. Data generated from these longitudinal studies were not suggestive of disease recurrence before other routine clinical tests had identified relapse. Therefore, we must conclude that these in vitro assays are not of significant prognostic value to the evaluation of patients with osteogenic sarcoma, although they may be so for certain carcinomas. Other immunologic assays are of value in correlating clinical tumor response with prognosis and deserve evaluation in this patient group.

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Sequential Combination Chemotherapy With Vinblastine-Bleomycin and Doxorubicin-*cis*-Dichlorodiammineplatinum(II) in Disseminated Nonseminomatous Testicular Cancer[1,2]

Max E. Scheulen,* Siegfried Seeber, Rudolf B. Schilcher, Carl R. Meier, and Carl G. Schmidt[3]

SUMMARY

Forty-two patients with disseminated nonseminomatous testicular cancer were randomly entered to receive a two-armed chemotherapy regimen with mandatory crossover, consisting of either vinblastine-bleomycin or doxorubicin-*cis*-dichlorodiammineplatinum(II) (DDP) as initial therapy. Forty of these patients received at least four courses and were considered evaluable. Twenty-five of these patients received vinblastine-bleomycin and 15 received doxorubicin-DDP as initial treatment. The overall remission rate was 88%, with a complete remission rate of 68%. Thirty-two patients remain alive and 20 (50%) remain disease-free at 3+ to 18+ months, with a median duration of complete remissions of 9+ months. The response rate was not affected by prior chemotherapy or radiotherapy. Either treatment arm proved equally effective. However, response with doxorubicin-DDP therapy occurred in nine of 19 treatment courses when vinblastine-bleomycin therapy had failed, while response with vinblastine-bleomycin therapy occurred in only two of 11 treatment courses when doxorubicin-DDP therapy had failed. Thus, different patterns of cross-resistance between these alternative regimens may exist.

[Cancer Treat Rep 64:599–609, 1980]

Disseminated testicular cancer is responsive to a variety of cytotoxic drugs, including vinblastine (1), bleomycin (2), *cis*-dichlorodiammineplatinum(II) (DDP) (3,4), and doxorubicin (Adriamycin) (5,6). By combining vinblastine and bleomycin, Samuels et al (7) achieved a 39% complete remission rate. Einhorn and Donohue (8) added DDP to a modified vinblastine-bleomycin combination and achieved a 74% complete remission rate. Since complete responders may require long-term chemotherapy, cumulative toxicity and eventual development of drug-resistance are the potential problems.

Although doxorubicin proved effective as a single drug (5,6), various drug combinations including doxorubicin (9–17) gave results not comparable to the regimen used by Einhorn and Donohue (8). On the other hand, doxorubicin in combination with DDP may be effective in patients refractory to vinblastine-bleomycin (18). The potential additive contribution of doxorubicin to the established efficacy of the vinblastine-bleomycin-DDP combination remained to be tested in a responsible fashion using effective doses while avoiding additive myelotoxicity. We report the results of a sequentially administered chemotherapy regimen consisting of vinblastine-bleomycin and doxorubicin-DDP in order to: (*a*) document efficacy of this four-drug combination; (*b*) define the relative contribution of either treatment arm to remission induction; (*c*) delay acquired drug resistance; (*d*) avoid cumulative toxicity; and (*e*) recognize patterns of cross-resistance.

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TABLE 1.—Patient characteristics

Characteristic	Initial therapy	
	Group A: vinblastine-bleomycin	Group B: doxorubicin-DDP
No. of evaluable patients	25	15
Primary tumor		
Testis	24	15
Extragonadal	1	0
Median age in yrs (range)	28 (19–43)	29 (15–47)
Median duration of disease in mos (range)	2 (0–64)	1 (0–25)
No. of patients with prior—		
Total retroperitoneal node dissection	9	6
Subtotal retroperitoneal node dissection	5	2
Radiotherapy	2	1
Chemotherapy	1	1
Radiotherapy and chemotherapy	3	1
No. of patients with—		
Minimal pulmonary disease	7	2
Advanced pulmonary disease	12	9
Advanced abdominal disease	6	4
Tumor type*		
II, embryonal carcinoma and seminoma	14	7
III, teratoma	1	4
IV, teratocarcinoma, mixed	7	4
V, choriocarcinoma with or without embryonal carcinoma	3	0
Tumor markers and LDH		
Elevated β-human chorionic gonadotropin	5	4
Elevated α-fetoprotein	1	4
Elevated β-human chorionic gonadotropin and α-fetoprotein	5	2
Elevated LDH	16	8

*Dixon and Moore classification (ref 19).

PATIENTS AND METHODS

Forty-two patients with nonseminomatous testicular cancer with measurable metastatic disease were entered in the study. The median age was 28 years with a range of 15–47 years. None of the patients had received prior chemotherapy with any of the drugs used in our regimen. Nine patients had been previously treated with cyclophosphamide, actinomycin D, methotrexate, ifosfamide, chlorambucil, and/or radiation therapy. Twenty-two patients had undergone retroperitoneal lymphadenectomy (table 1).

Forty patients received at least four courses and were considered evaluable. Two patients died within the first 2 months after initiation of chemotherapy. Patients were randomly assigned to either treatment group A or B (fig 1). Group A consisted of 25 evaluable patients who received vinblastine-bleomycin as initial therapy as described by Samuels et al (7). Vinblastine was administered at a total dose of 0.4 mg/kg *split* into two injections on Days 1 and 2. Thirty units of bleomycin diluted in 1000 ml of normal saline was given daily by continuous iv infusion over a 24-hour period for 5 consecutive days (Days 1–5). Group B consisted of 15 evaluable patients who received doxorubicin-DDP as initial therapy, namely, doxorubicin at a dose of 60 mg/m² iv on Day 1 and DDP at a dose of 20 mg/m²/day for 5 consecutive days (Days 1–5) as a 15-minute iv infusion, with continuous iv infusion of 2500 ml/24 hours of normal saline.

Patients were maintained on the treatment plan (fig 1) for at least 12 courses unless major toxicity or progression of disease made a change in chemotherapy necessary. If, after each two evaluable courses of both chemotherapy arms only one combination had proved to be effective, this was chosen for further treatment until failure. If complete response was not achieved by either combination, alternative chemotherapy combinations were used such as ifosfamide plus VP-16-213. The dose schedule was 40 mg/kg iv daily for 5 consecutive days for ifosfamide and 120 mg/m² orally daily for 5 consecutive days for VP-16-213.

All patients in the study had pulmonary involvement. According to extent and location of disease, they were divided into tumor status groups as follows (modified after Samuels et al [7]).

(*a*) Minimal pulmonary disease, ie, total tumor volume of < 10 cm³ as estimated by standard chest roentgenogram.

(*b*) Advanced pulmonary disease, ie, any mediastinal or hilar mass, pleural effusion, or total intrapulmonary tumor volume of > 10 cm³.

(*c*) Advanced abdominal disease, ie, palpable mass, liver metastases, ureteral displacement, distortion of inferior vena cava, or advanced abdominal disease found at laparotomy.

Patients were monitored by serial chest roentgenograms, abdominal ultrasonography, and measurements of β-human chorionic gonadotropin titers as well as levels of α-fetoprotein, LDH, and alkaline phosphatase. Iv pyelography, liver scintiscanning, chest roentgenogram-tomography, and computerized axial tomography provided additional information when indicated. Patients who achieved sta-

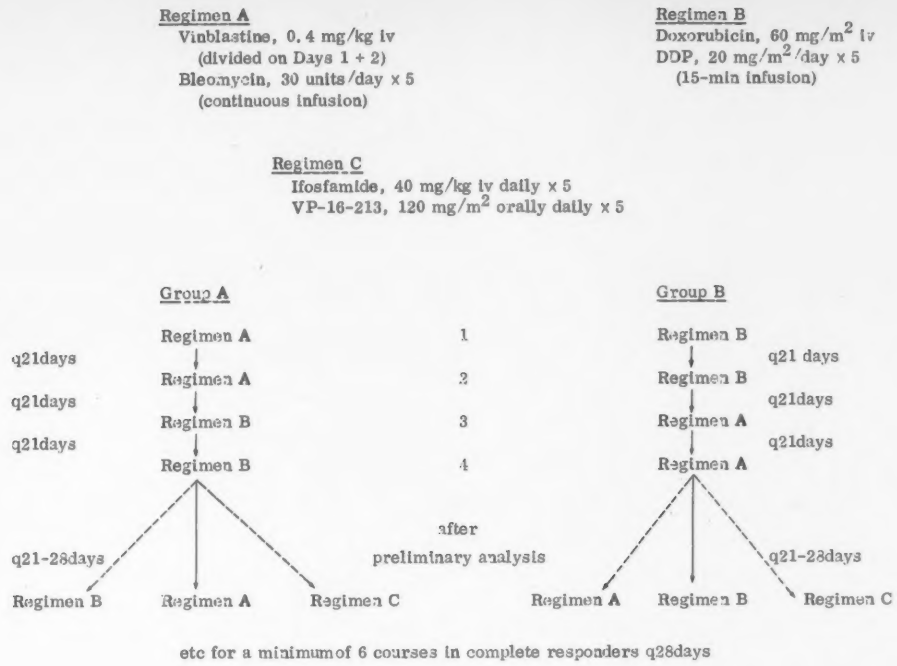

FIGURE 1.—Treatment plan.

ble partial remission or had no change of disease were considered for thoracotomy or laparotomy after 12 courses of chemotherapy.

The histologic classification of Dixon and Moore (19) was used. Objective tumor regression was graded as follows: *complete remission*—the disappearance of all measurable tumor and the normalization of all laboratory parameters listed above for at least 2 months; and *partial remission*—at least 75% decrease in total tumor volume.

RESULTS

Response Rates

The results for both arms of induction chemotherapy in stage III nonseminomatous testicular cancer are listed in tables 2–6. The overall complete response rate was 68% for all patients (table 4). There was no difference in response rate between those patients who initially received vinblastine-bleomycin or those who received doxorubicin-DDP: 17 of the 25 patients initially treated with vinblastine-bleomycin achieved complete remission, and ten of these 17 patients had no evidence of disease after two courses (table 2). Conversely, ten of the 15 patients who initially received doxorubicin-DDP had a complete response, and six of these ten patients had no evidence of disease after two courses (table 3). When responses were analyzed according to status of disease at initial presentation, patients with minimal pulmonary disease had the best prognosis, with no recurrences thus far registered in this group (table 5). Patients with advanced abdominal disease who were considered complete responders (five of ten patients = 50%) had a high recurrence rate (three of five patients) (fig 2). In an actuarial survival analysis (fig 3), the median survival time of the complete responders had not been reached at 18 months, while the group of nonresponders plus partial responders had a median survival time of 10 months.

Patterns of Cross-Resistance

An analysis for cross-resistance between vinblastine-bleomycin and doxorubicin-DDP made it necessary to evaluate each individual course of this study. In this setting it seemed reasonable to distinguish between effective and noneffective chem-

TABLE 2.—Clinical data of individual patients initially treated with vinblastine-bleomycin (group A)

Patient No.	Age (yrs)	Tumor* type	Prior therapy†	Disease status upon entry in the study: Pulmonary: Volume (cm^3)	Pulmonary: No. of metastases	Extrapulmonary	Response	Time to response (No. of courses of chemotherapy)	Duration of response (mos)	No. of courses of chemotherapy	Survival (mos)	Present status
1	19	II	OE, and total RND	1.1	6		Complete	1	18+	12	18+	Well
2	20	IV	OE	1.2	2	Positive lymphangiogram	Complete	1	10+	11	11+	Well
3	29	II	OE, radiotherapy	1.7	3		Complete	2	8+	10	9+	Well
4	27	II	OE, total RND	1.9	3		Complete	2	6+	8	7+	Well
5	24	II	OE, subtotal RND	3.6	3	Positive lymphangiogram	Complete	4	9+	12	12+	Well
6	24	V	OE, total RND	15	5		Complete	5	3+	8	6+	Well
7	36	IV	OE, total RND	23	3		Complete	2	9+	9	10+	Well
8	39	II	OE, total RND, and chlorambucil, methotrexate, actinomycin D	24	6	Nonfunctioning right kidney	Complete	2	9+	12	11+	Well
9	38	III	OE	36	4	Positive lymphangiogram, mediastinal mass, supraclavicular nodes	Complete	2	3	11	12	Died of pulmonary and abdominal disease
10	40	II	OE, radiotherapy, cyclophosphamide	38	5	Positive lymphangiogram	Complete	1	10+	11	11+	Well
11	25	IV	OE, total RND, left nephrectomy	39	6		Complete	2	3+	4	4+	Well
12	28	II	OE	40	8	Positive lymphangiogram	Complete	4	3+	7	5+	Well
13	41	IV	OE	110	39	Positive lymphangiogram	Complete	4	3+	8	6+	Well
14	26	II	OE, total RND, bilateral ureteral splint	360	5		Complete	4	10+	12	13+	Well
15	43	IV	Subtotal RND, radiotherapy, cyclophosphamide	19	1	Extragonadal primary tumor, ureteral displacement	Complete	2	6	11	14+	Abdominal relapse, alive

16	28	II	OE, subtotal RND, radiotherapy, cyclophospha-mide	42	18	Palpable abdominal mass, supraclavicular nodes	Complete	4	3	10	9+	Pulmonary relapse, alive
17	19	V	OE	150	22	Ureteral displacement, liver metastases	Complete	2	3	7	6	Died of pulmonary and hepatic disease
18	22	II	OE, subtotal RND	1.6	2	Positive lymphangiogram	Partial	—	4	5	4+	Pulmonary disease
19	39	II	OE, total RND	35	31		Partial	—	4+	4	4+	Pulmonary disease, improving
20	27	II	OE	130	10	Positive abdominal ultrasonogram	Partial	—	5+	6	5+	Pulmonary disease, improving
21	36	V	OE	29	12	Distortion of inferior vena cava	Partial	—	4	10	9	Died of pulmonary and abdominal disease
22	21	II	OE	120	11	Ureteral displacement, supraclavicular nodes	Partial	—	8	10	10	Died of pulmonary and hepatic disease
23	36	IV	OE	120	7	Ureteral displacement	Partial	—	5	9	10	Died of pulmonary and abdominal disease
24	36	II	OE, total RND	1.1	6		No change	—	—	9	8+	Pulmonary disease
25	42	IV	OE, subtotal RND	23	3	Positive lymphangiogram	Disease progression	—	—	10	11	Died of pulmonary and abdominal disease

*Dixon and Moore classification (19).
†OE = orchiectomy; RND = retroperitoneal node dissection.

TABLE 3.—Clinical data of individual patients initially treated with doxorubicin-DDP (group B)

Patient No.	Age (yrs)	Tumor* type	Prior therapy†	Disease status upon entry in the study: Pulmonary: Volume (cm^3)	Pulmonary: No. of metastases	Extrapulmonary	Response	Time to response (No. of courses of chemotherapy)	Duration of response (mos)	No. of courses of chemotherapy	Survival (mos)	Present status
1	23	II	OE, radiotherapy, actinomycin D	1.2	1	Positive lymphangiogram, supraclavicular nodes	Complete	1	9+	9	9+	Well
2	18	IV	OE	4.2	1		Complete	1	10+	11	11+	Well
3	29	II	OE	14	3	Positive lymphangiogram	Complete	4	3+	7	6+	Well
4	31	III	OE, total RND	15	12		Complete	1	15+	12	16+	Well
5	26	II	OE, subtotal RND	36	6	Abdominal tumors at laparotomy, supraclavicular nodes	Complete	2	3	7	6+	Pulmonary relapse, alive
6	35	IV	OE, total RND	38	23		Complete	4	2	12	12+	Pulmonary relapse, alive
7	15	II	OE, total RND	43	12		Complete	2	8+	9	9+	Well
8	25	II	OE	160	31		Complete	5	3(+)	9	9	Died of complications of chemotherapy in complete remission
9	22	III	OE	3.4	3	Ureteral displacement	Complete	2	5+	7	6+	Well
10	36	II	OE, total RND, ifosfamide	69	5	Ureteral displacement	Complete	3	8+	10	10+	Well
11	26	III	OE, radiotherapy	49	5	Positive lymphangiogram	Partial	—	10	9	14+	Thoracotomy: mature teratoma
12	31	II	OE, total RND	180	1	Mediastinal tumor, supraclavicular nodes	Partial	—	4+	5	4+	Pulmonary disease, improving
13	32	IV	OE, total RND	35	2	Mediastinal tumor	Disease progression	—	—	10	11+	Pulmonary disease, progression
14	47	IV	OE, subtotal RND	6.0	6	Liver metastases at laparotomy	Disease progression	—	—	11	11+	Pulmonary disease, progression
15	38	III	OE	370	18	Liver metastases	Disease progression	—	—	10	10	Died of pulmonary disease

*Dixon and Moore classification (19).
†OE = orchiectomy; RND = retroperitoneal node dissection.

TABLE 4.—Response rates

Type of response	No. of patients (%)* responding to initial treatment in—		
	Group A: vinblastine-bleomycin	Group B: doxorubicin-DDP	Groups A + B
Complete remission	17 (68)	10 (67)	27 (68)
Partial remission	6 (24)	2 (13)	8 (20)
No change	1 (4)	—	1 (2)
Disease progression	1 (4)	3 (20)	4 (10)

*Total No. of evaluable patients: group A = 25, group B = 15, groups A + B = 40.

TABLE 5.—Response by presentation

Disease status at initial presentation	Total No. of patients	No. of complete responders (%)	No. of patients in complete remission at present (%)
Minimal pulmonary disease	9	7 (78)	7 (78)*
Advanced pulmonary disease	21	15 (71)	12†(57)
Advanced abdominal disease	10	5 (50)	2 (20)*

*$P < 0.05$.

†Including 1 patient in complete remission who died of chemotherapy complications.

TABLE 6.—Patterns of cross-resistance

Chemotherapy	Cumulated No. of evaluable courses of chemotherapy	No. of disease regressions (%)	No. of evaluable courses following failure of alternative combination	No. of disease regressions (%)
Regimen A				
Vinblastine-bleomycin	72	49 (68)	11	2 (18)
Regimen B				
Doxorubicin-DDP	64	47 (73)	19	9 (47)

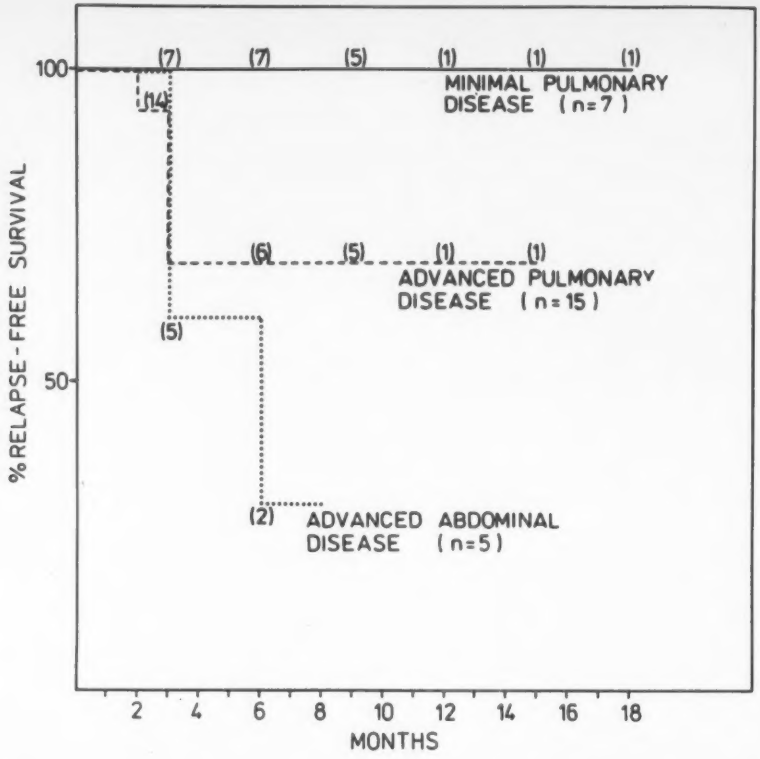

FIGURE 2.—Actuarial relapse-free survival of complete responders with stage III nonseminomatous testicular cancer according to disease status at initial presentation. *Minimal pulmonary disease*—100% ± 0 at 18 mos; *advanced pulmonary disease*—69% ± 13% at 15 mos; *advanced abdominal disease*—30% ± 24% at 8 mos.

otherapy courses only. Effective courses were defined when objective response was measurable (> 10% tumor regression/course); ineffective courses included no response (stable disease) or progression. Chemotherapy courses in patients with complete response who had no evidence of disease were not evaluated. Seventy-two individual courses of vinblastine-bleomycin and 64 courses of doxorubicin-DDP were considered evaluable according to this definition (table 6). Regression was measurable in 49 of 72 courses of vinblastine-bleomycin (68%) compared to 47 of 64 courses of doxorubicin-DDP (73%). Thus, it was shown that not only the sequential regimens A and B (fig 1) but also the chemotherapy programs for groups A and B (table 4) were equally effective in inducing remissions. When doxorubicin-DDP therapy had failed, 11 subsequent courses of vinblastine-bleomycin were evaluable for response. Only two of these 11 courses (18%) were effective, with a minimum of 10% regression/course. Conversely, doxorubicin-DDP courses following disease progression with vinblastine-bleomycin therapy were effective in nine of 19 evaluable courses (47%) (table 6). With the combination of ifosfamide and VP-16-213, responses were achieved in about 40% of all evaluable courses given to patients refractory to vinblastine-bleomycin and doxorubicin-DDP, but no additional complete responses were achieved.

Toxicity

Most patients had myelosuppression, but the wbc count nadirs were $< 10^3$ cells/mm^3 in only five patients. One patient died from septicemia while in complete remission (No. 8, table 2). Serum creatinine elevations due to DDP nephrotoxicity were minimal (median, 1.1 ± 0.2 mg/100 ml; maximum, 1.6 mg/100 ml). No cardiotoxicity episodes were registered thus far with a cumulated dose of dox-

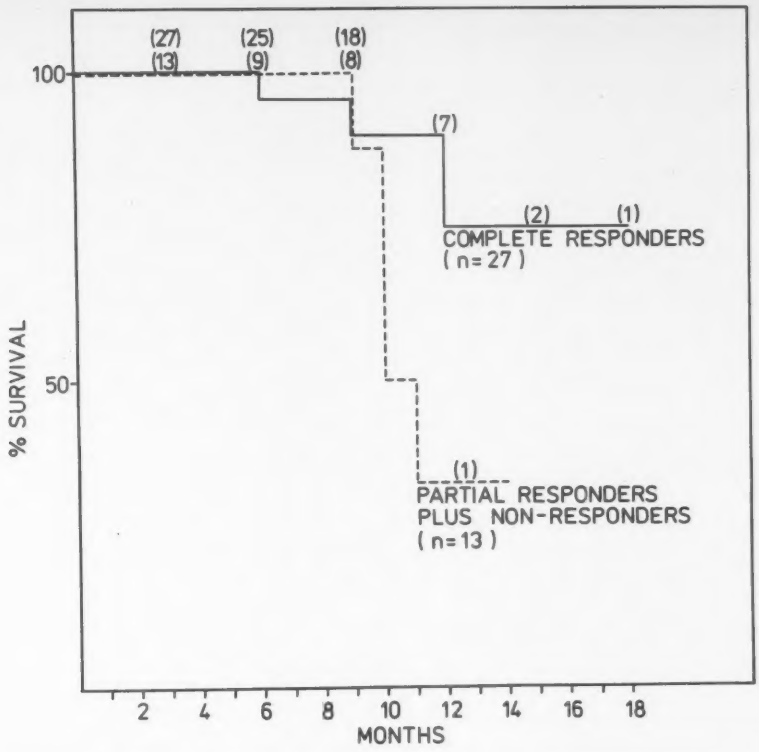

FIGURE 3.—Actuarial survival of 27 complete responders and 13 nonresponders + partial responders with stage III nonseminomatous testicular cancer. *Complete responders*—75% ± 17% at 18 mos; *nonresponders + partial responders*—33% ± 18% at 14 mos.

orubicin limited to 360 mg/m². Pulmonary fibrosis was clinically suspected in two patients but could not be biopsy-proven.

DISCUSSION

Complete response rates between 50% and 70% have become the rule in the chemotherapy for stage III nonseminomatous testicular cancer (8,17,20–24). The median duration of such remissions is far beyond 1 year in most series, and, accordingly, the median survival time of complete responders has not been reached in a number of recent studies. The data reported here appear to match well with these figures and suggest a significant cure rate of somewhere between 25% and 50% of all stage III patients. The response to induction chemotherapy is closely related to the status of disease at initial presentation. As reported earlier by Samuels et al (25), the prognostic difference between patients with minimal pulmonary disease and patients with advanced abdominal disease has also been true for the patients of our series at a high level of significance. In contrast, the histologic subtype appears to be less important with respect to prognosis, and no histology-related drug sensitivity seems to exist when responses of embryonal carcinomas and teratocarcinomas are compared.

Valuable new information derived from our study includes (*a*) the high effectiveness of the doxorubicin-DDP combination in patients without prior extensive treatment, (*b*) the efficacy of a sequential approach with vinblastine-bleomycin and doxorubicin-DDP irrespective of the primary sequence of these two combinations, and (*c*) the low level of cross-resistance of the doxorubicin-DDP combination when applied in patients refractory to or progressive under vinblastine-bleomycin. Based on earlier studies of the doxorubicin-DDP combination in five small series showing a total of only five of

26 complete responses, the therapeutic contribution of doxorubicin-DDP may have been underestimated (13–16,18).

The advantages of this protocol have been the low toxicity and the easy handling of both two-drug regimens, and it was found desirable to individualize treatment after four courses, ie, two of each regimen, when the less effective combination could be omitted. It appears logical, however, to emphasize the importance of the induction chemotherapy period which should lead to an early complete remission in as many patients as possible. Most of the possibly different tumor cell clones of malignant teratomas should be sensitive to at least one or two of the four drugs applied in this protocol within the first 3 months. Further improvement may be achieved by leukocyte nadir-related dose adaptation and by an individualized close spacing of the initial chemotherapy courses.

Such measures as well as surgery after chemotherapy may be an approach that is highly likely to improve the relatively poor results in patients with advanced abdominal disease. The question of the optimal duration of such treatment is still unsolved. From recent observations, fairly short periods of chemotherapy have been suggested (24). However, the duration of chemotherapy should probably be decided individually, depending on the time until a complete remission has been reached. Since cumulative toxicity as well as acute complications have been found low with this sequential protocol, it has also been used in stage II nonseminomatous testicular cancer following dissection of retroperitoneal lymph nodes in an adjuvant fashion. We have recently reported on actuarial 3-year survival rates of close to 90% for early stage II patients (26) indicating that sequential combination chemotherapy with non-cross-resistant programs also favorably affects the prognosis in stage II patients. Thus, sequential combination chemotherapy with vinblastine-bleomycin and doxorubicin-DDP appears to be an effective chemotherapeutic modality in early and advanced nonseminomatous testicular cancer.

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Effect of Sodium Thiosulfate on *cis*-Dichlorodiammineplatinum(II) Toxicity and Antitumor Activity in L1210 Leukemia[1,2]

Stephen B. Howell* and Raymond Taetle[3]

SUMMARY

Concurrent administration of sodium thiosulfate reduced the toxicity of *cis*-dichlorodiammineplatinum(II) (DDP) in a dose-related manner in mice. Sodium thiosulfate protected mice against an otherwise lethal dose of DDP (20 mg/kg), and reduced DDP-induced weight loss. Sodium thiosulfate (800 mg/kg) injected within 1 hour before or ½ hour after DDP blocked nephrotoxicity as measured by a rise in BUN, an increase in kidney weight, and medullary hemorrhage. In culture, sodium thiosulfate markedly reduced the toxicity of DDP to mouse colony-forming units. Concurrent injection of sodium thiosulfate partially reduced the antitumor activity of DDP. The therapeutic dose range of DDP was expanded, but the maximum increase in lifespan of mice bearing L1210 leukemia was reduced by 40%. Sodium thiosulfate offers the possibility of systemic protection against the cytotoxicity of regionally administered DDP in man.

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The *cis*-dichlorodiammineplatinum(II) complex (DDP) forms cross-links between macromolecules primarily at the sites of nucleophilic amino groups (1,2). Interstrand cross-links in DNA account for a major part of DDP cytotoxicity to rapidly proliferating cells (3–5), but DNA-protein complexes and binding to other macromolecules may also contribute to cell damage. The mechanism of toxicity to slowly proliferating tissues such as the kidney is less understood. The prominent nephrotoxicity of this agent may be due to the linking of the platinum coordination complex to macromolecules, but the renal tubular epithelium is particularly sensitive to free heavy metal salts, and the extent to which renal toxicity results from either of these two mechanisms has not been defined. In addition to binding to nucleophilic amino groups, DDP also binds readily to some sulfur-containing analogs. Co-administration of thiourea, but not cysteine or *N*-acetylcysteine, reduces the toxicity and antitumor activity of DDP (6–9). Thiourea under some in vitro conditions can dissociate established DNA interstrand cross-links and reverse the lethal lesions caused by DDP (8). These observations raise the possibility of selective rescue of DDP (7), or selective protection of the kidneys from large doses of DDP, with sulfur-containing compounds that concentrate in the urine.

This report describes the effect of another sulfur-containing compound, sodium thiosulfate, on the toxicity and antitumor activity of DDP. Sodium thiosulfate was chosen because it is readily available for iv use in man, and has a record of safety even when used in large doses in patients poisoned with cyanide.

MATERIALS AND METHODS

Experiments were carried out in 20–25-g B6D2F$_1$ male mice obtained from The Jackson Laboratories (Bar Harbor, ME). Both DDP and sodium thiosulfate were injected via the ip route; when both drugs were administered together they were injected

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from separate syringes into different parts of the peritoneal cavity. For studies of nephrotoxicity, mice were bled from the retro-orbital sinus 72 hours after drug exposure, and BUN was measured on serum samples by the urease technique (10). In chemotherapy studies, groups of six mice were inoculated with 10^6 L1210 cells ip on Day 0. Treatment was administered on Day 1, and mice were monitored for median day of death. DDP was obtained from the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). It was reconstituted just before use to form a stock solution of DDP (1 mg/ml), NaCl (9 mg/ml), and mannitol (10 mg/ml). Sodium thiosulfate of analytic reagent grade was obtained from Mallinckrodt and stored as a stock solution of $Na_2S_2O_3$ (100 mg/ml) in 0.9% NaCl at 4°C.

The assay for murine colony-forming units in culture (CFU-C) was performed using a modification of techniques described elsewhere (11). Femoral marrow was removed by flushing the femur with sterile McCoy's 5A medium. One million washed marrow cells were exposed to various concentrations of DDP in the presence or absence of sodium thiosulfate (0.1 mg/ml) for 1 hour. The cells were then washed and plated at a concentration of 5×10^4 cells/ml over wbc feeders in McCoy's 5A medium containing 0.8% methylcellulose and 15% fetal calf serum (FCS). Wbc feeders were prepared in McCoy's 5A medium containing 0.5% agar and 15% FCS, plus 10^6 human peripheral blood leukocytes as described by Pike and Robinson (12). Aggregates of > 50 cells were scored as colonies by inverted microscope after a 7-day incubation at 37°C in 7.5% CO_2.

RESULTS

A single ip dose of DDP (20 mg/kg) reproducibly caused nephrotoxicity in BDF_1 mice, and killed 80%–100% of the mice by Day 6 after injection. Nephrotoxicity was manifested by a rise in the BUN from the normal range of 17 ± 2 to 95 ± 32 mg/dl, and the appearance of hemorrhagic changes in the renal medulla within 72 hours. Sodium thiosulfate was found to reduce the toxic effects of DDP when both drugs were injected concurrently. Figure 1 shows the effect of a single injection of sodium thiosulfate on DDP-induced nephrotoxicity quantitated by measurement of BUN 72 hours after DDP administration. Both drugs were given ip; the sodium thiosulfate was injected immediately after the DDP. A dose of 400 mg/kg of sodium thiosulfate reduced nephrotoxicity by approximately 50%, whereas 800 mg/kg of sodium thiosulfate afforded

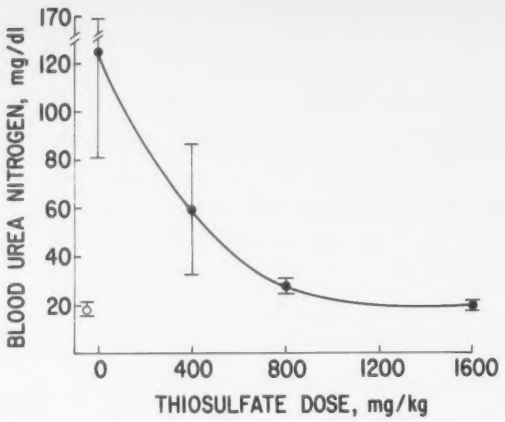

FIGURE 1.—Effect of sodium thiosulfate dose on DDP-induced nephrotoxicity. Serum BUN was measured 72 hrs after ip injection of DDP (20 mg/kg) alone or followed immediately by sodium thiosulfate in increasing doses (● — ●); uninjected controls (0). Each point = mean ± SD of measurements made in 5 mice.

nearly complete protection. Figure 2 compares the gross appearance of kidneys from representative mice treated with DDP (20 mg/kg) with or without sodium thiosulfate (800 mg/kg) concurrently. Extensive medullary hemorrhage was evident in the kidneys from the mice treated with DDP alone. The ratio of the combined weight of the kidneys to total body weight at 72 hours was 0.007 ± 0.001 for mice treated with DDP alone, and 0.005 ± 0.0001 for mice treated with DDP plus sodium thiosulfate ($P < 0.001$); the latter value is not significantly different from that for untreated mice.

The ability of sodium thiosulfate to reduce DDP-induced weight loss is shown in figure 3. Mice receiving DDP (20 mg/kg) alone lost 30% of their initial body weight by Day 4. Sodium thiosulfate doses of 400, 800, and 1600 mg/kg injected immediately after DDP afforded progressively greater protection, resulting in Day-4 weight losses of 27%, 21%, and 9%, respectively. Sodium thiosulfate also protected against the lethal effect of DDP. In the representative experiment shown in figure 3, only one of five mice receiving DDP (20 mg/kg) alone survived beyond Day 6. In contrast, there was only one toxic death among the five mice receiving DDP plus 400 mg/kg of sodium thiosulfate, and all mice receiving 800 and 1600 mg/kg of sodium thiosulfate recovered completely. Sodium thiosulfate itself caused no discernible toxic effects in single doses ≤ 3200 mg/kg ip.

The ability of sodium thiosulfate to block the toxicity of DDP to mouse marrow was examined using

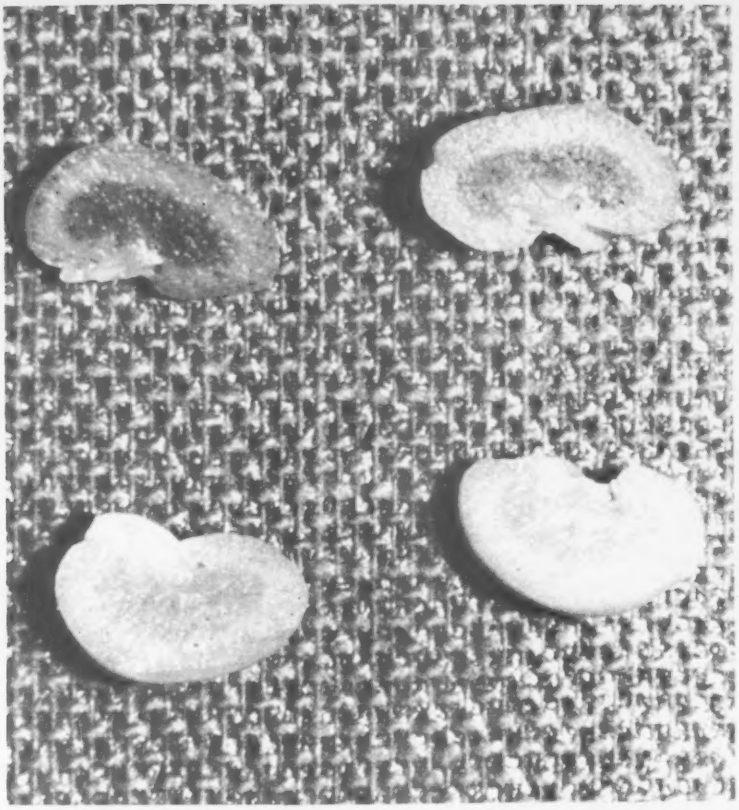

FIGURE 2.—Longitudinal hemisections of representative kidneys from mice treated with either DDP alone (upper) or DDP + sodium thiosulfate (lower). DDP (20 mg/kg) ± sodium thiosulfate (800 mg/kg) was injected ip 72 hrs before mice were killed. Note the presence of medullary hemorrhage in the kidneys of mice that did not receive sodium thiosulfate protection.

an in vitro assay of CFU-C. Figure 4 demonstrates that sodium thiosulfate itself was toxic to CFU-C colonies only when present at a dose of 10 mg/ml. Figure 5 shows that sodium thiosulfate at a dose of 0.1 mg/ml significantly reduced the toxicity of DDP over the entire concentration range of 0–10 μg/ml, and that protection was almost complete up to a DDP concentration of 5 μg/ml.

The temporal aspects of sodium thiosulfate protection against nephrotoxicity were explored using doses of 800 mg/kg at various times before and after 20 mg/kg of DDP. Nephrotoxicity was quantitated by measurement of BUN at 72 hours after injection, and both drugs were injected ip at separate sites. Figure 6 shows that sodium thiosulfate was effective only during a rather narrow window of time extending from 1 hour before DDP to ½ hour after DDP. Administration of sodium thiosulfate earlier preceding DDP, or later following DDP, failed to have any effect on the subsequent rise in BUN.

To determine whether there was a direct interaction between DDP and sodium thiosulfate, or whether the sodium thiosulfate was indirectly altering the sensitivity of normal tissues to DDP, various doses of DDP were combined with 800 mg/kg of sodium thiosulfate in the same syringe immediately before injection. Under these conditions, DDP in doses ≤ 20 mg/kg caused no toxicity in the form of weight loss or rise in BUN (data not shown).

The effect of sodium thiosulfate on the antitumor activity of DDP was examined in the L1210 leukemia system using a single dose of DDP (5–45 mg/kg) administered 1 day after tumor inoculation. In these studies, a single dose of 800 mg/kg of sodium

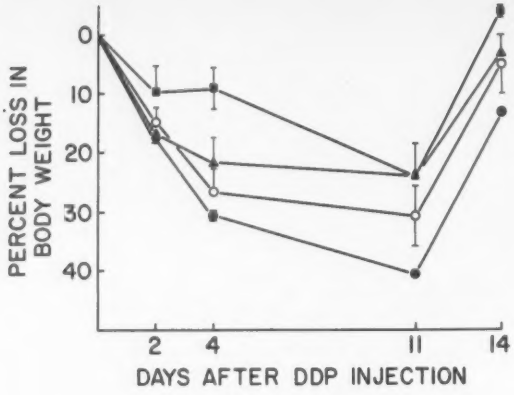

FIGURE 3.—% loss of body weight in mice receiving DDP (20 mg/kg) ip alone (● — ●), or in combination with ip sodium thiosulfate at 400 mg/kg (○ — ○), 800 mg/kg (▲ — ▲), or 1600 mg/kg (■ — ■). Each point = mean ± SD of measurements made in 5 mice, except that in the DDP-alone group only 1 animal survived beyond Day 6.

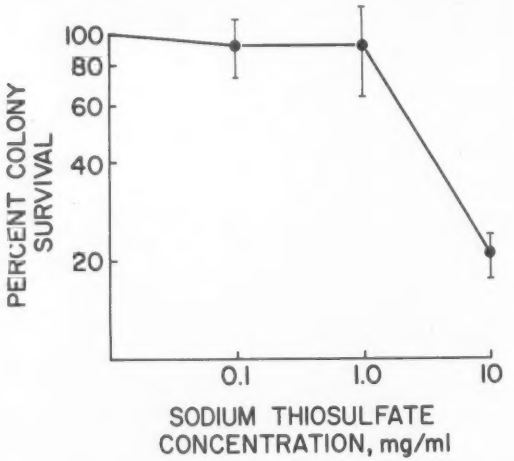

FIGURE 4.—% of control marrow CFU-C survival produced by various concentrations of sodium thiosulfate. The sodium thiosulfate was left in contact with marrow cells during the whole period of colony formation. Each point = mean ± SD of triplicate plates.

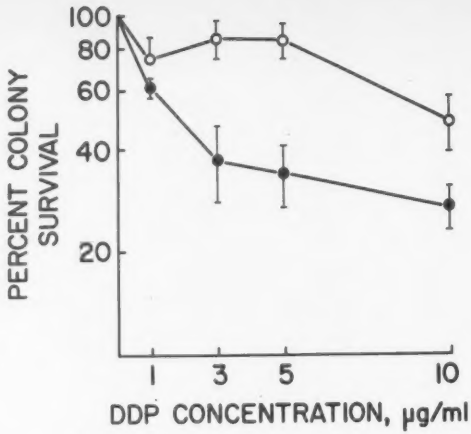

FIGURE 5.—% of control marrow CFU-C survival in the presence or absence of various concentrations of either DDP alone (● — ●) or DDP + 0.1 mg/ml of sodium thiosulfate (○ — ○). Drugs remained in contact with marrow cells during the whole period of colony formation. Each point = mean ± SD of triplicate plates.

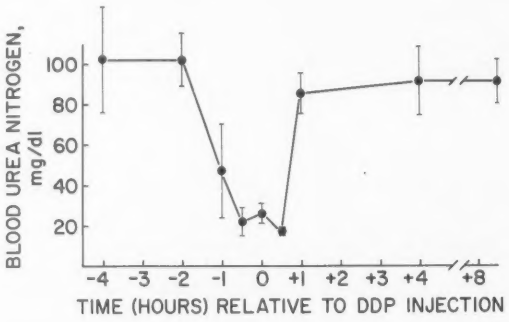

FIGURE 6.—Effect of time of injection of sodium thiosulfate relative to administration of DDP on protection against DDP-induced nephrotoxicity. Sodium thiosulfate (800 mg/kg) was injected ip at various times before and after ip injection of DDP (20 mg/kg). Serum BUN was measured 72 hrs after DDP administration. Each point = mean ± SD of measurements made in 5 mice. The mean BUN of uninjected controls was 18 ± 1 mg/dl.

thiosulfate was given immediately after the DDP, and both drugs were injected ip. As shown in figure 7, DDP used alone produced a peak increase in lifespan of 50% at a dose of 10 mg/kg, with control mice surviving 7–9 days; the therapeutic range was narrow and doses of ⩾ 20 mg/kg were toxic. The concurrent injection of sodium thiosulfate reduced both the toxicity and the antitumor activity of DDP. Doses of DDP as high as 30 mg/kg were tolerated and the therapeutic dose range was expanded, but the maximum increase in lifespan was only 30%. In the experiments shown in figure 7, 800 mg/kg of sodium thiosulfate reduced the antitumor activity of 5 mg/kg of DDP by two thirds; in other trials (data not shown), sodium thiosulfate in doses of ⩾ 1600 mg/kg completely blocked all antitumor activity of this dose of DDP.

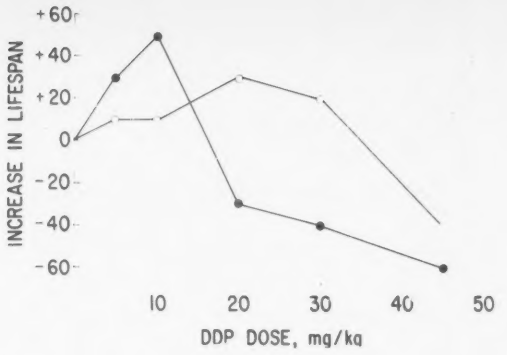

FIGURE 7.—Effect of increasing doses of DDP alone (● — ●), or DDP followed immediately by 800 mg/kg of sodium thiosulfate (○ — ○), on the lifespan of mice bearing early L1210 leukemia. Both drugs were injected concurrently at separate ip sites.

DISCUSSION

DDP has emerged as an important chemotherapeutic agent with activity in a wide variety of human neoplasms. The dose-limiting toxic effect is damage to the renal tubular epithelium. Even partial protection of the kidney with an agent that does not also reduce the antitumor activity of DDP may allow more frequent dose scheduling and higher response rates. Sulfur analogs readily serve as targets for nucleophilic attack by DDP, and several sulfur-containing compounds are capable of blocking the formation of DDP-DNA complexes (8) and reducing the cytotoxicity of DDP (7,13). Sodium thiosulfate was selected from among these compounds because of its relative safety and ready availability for use in man.

This study indicates that sodium thiosulfate produces a dose-related decrease in the toxicity of DDP in the mouse. This was reflected by a reduction in weight loss, nephrotoxicity, and marrow CFU-C toxicity and by increased survival after an otherwise lethal dose of DDP. At a molar dose ratio of 75:1, a dose of DDP threefold greater than the optimal therapeutic dose was still not toxic when sodium thiosulfate was injected concurrently. A sodium thiosulfate-to-DDP concentration ratio of 38 was sufficient to abrogate a > 60% reduction in marrow CFU-C caused by DDP alone. Sodium thiosulfate itself was not toxic in vivo at doses four times greater than that required to completely block the toxicity of DDP, and demonstrated no toxic effects to mouse marrow CFU-C in vitro at concentrations > 100-fold higher than the effective neutralizing concentration. Unfortunately, when both drugs were injected simultaneously into the peritoneal cavity, sodium thiosulfate also reduced the antitumor activity of DDP against the L1210 leukemia. Nonetheless, these results demonstrate several important features of the pathophysiology of DDP-induced nephrotoxicity and have implications for the use of protective agents in man. The observation that sodium thiosulfate failed to block nephrotoxicity when administered > 1 hour before DDP suggests rapid clearance of the sodium thiosulfate from the kidney. Lack of renal protection when sodium thiosulfate injection followed that of DDP by > ½ hour indicates that in vivo the damage done to the kidneys occurred very quickly, and that once established it was not reversible with the doses of sodium thiosulfate used in these experiments. This is consistent with the very rapid association (< 5 minutes) between DDP and renal tubular cells reported by Slater et al (9). Burchenal et al (7) reported that thiourea reduced the overall toxicity even when injected 2 hours after DDP administration. In the study reported here, the time frame during which sodium thiosulfate afforded significant renal protection was somewhat shorter (–1 to +½ hour). However, nephrotoxicity and lethal toxicity are not entirely synonymous. In vitro thio compounds can reverse established DDP-induced cross-links in DNA to some extent (8), but high concentrations (1 M) for relatively long periods of time (24 hours) are required. The fact that mixing the sodium thiosulfate with DDP prior to injection resulted in a completely inactive compound is consistent with the hypothesis that sodium thiosulfate acted to reduce toxicity by combining directly with DDP to form a complex no longer capable of cross-linking macromolecules, rather than by displacing DDP that was already bound.

An ideal protective agent would be one that reached only very low concentrations in the environment of the tumor but was extensively concentrated in the urine, and remained in the kidney until the renal excretion of active DDP was complete. Although urinary excretion of platinum after a single iv injection continues for several days (14), protection by a sulfur-containing analog may be needed only for several hours. Recent pharmacokinetic studies (15) suggest that while DDP itself is not protein-bound, DDP is rapidly metabolized by aquation to products that are tightly protein-bound, so that within the first 4 hours after injection the rate of delivery of free DDP to the kidney may decrease by more than two orders of magnitude. Sodium thiosulfate probably is not an ideal protective agent since it quickly equilibrates with extracellular water, and is rapidly metabolized and excreted (16). Sodium-2-mercaptoethane blocks the genitourinary

toxicity of cyclophosphamide (17), a drug whose mechanism of action is similar to that of DDP. Sodium-2-mercaptoethane is reported to reach high concentration in the urine without extensive uptake in other tissues (17) and thus may be a better protective agent for the kidney than sodium thiosulfate.

Irrespective of whether a sulfur-containing compound reduces the antitumor activity as well as the nephrotoxicity of DDP, if the distribution of protective agent from plasma into third-space fluids is limited, such compounds should provide an effective means of inactivating DDP reaching the systemic circulation after intracavitary DDP administration. Burchenal et al (7) demonstrated that the effectiveness of DDP injected ip in mice bearing L1210 leukemia was substantially enhanced when a sulfur-containing compound (thiourea) was introduced into the systemic circulation via the sc route. This approach may have particular relevance to the ip treatment of ovarian carcinoma, and the regional use of DDP chemotherapy with concurrent protection of the systemic circulation by this compound merits further study in man.

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Initial Clinical Study With *N*-(Phosphonacetyl)-L-aspartic Acid (PALA) in Patients With Advanced Cancer[1,2,3]

Ronald D. Hart,* Takao Ohnuma, and James F. Holland[4,5]

SUMMARY

Thirty-seven patients with inoperable malignancies were given 75 courses of *N*-(phosphonacetyl)-L-aspartic acid (PALA). Twenty-seven of these patients received PALA as a continuous iv infusion over 24 hours at dose levels ranging from 500 to 10,500 mg/m² of estimated body surface area. In addition, ten patients were given PALA by continuous iv infusion over 120 hours at total doses ranging from 4000 to 8700 mg/m². The dose-limiting toxic effects occurred in the skin (erythema, vesiculation, and bullae) and gastrointestinal mucosa (oral pain, cheilosis, oral mucosal ulceration, diarrhea, and hematochezia). Toxic reactions seemed more pronounced in patients with third-space fluid compartments. Myelosuppression was severe only in patients with pre-existing marrow dysfunction from neoplastic infiltration. No renal, hepatic, cardiac, or neurologic toxicity was seen. No cumulative toxic effects were evident in 14 patients who received repeated courses of PALA at 21-day intervals. For patients without third-space fluid, 8700 mg/m²/24 hours or 6500 mg/m²/120 hours were well-tolerated doses. One patient with chondrosarcoma had a partial response lasting 5 months.

[Cancer Treat Rep 64:617–624, 1980]

Based upon the proposal that enzymes bind most tightly to an "activated" intermediate form of their substrates (1–5) and that an analog of the transition-state intermediate would be a potent enzyme inhibitor (6,7), Collins and Stark (7) synthesized *N*-(phosphonacetyl)-L-aspartic acid (PALA) as a transition-state inhibitor of aspartate transcarbamylase (ATCase), an early enzyme in the de novo biosynthesis of pyrimidine nucleotides.

In preclinical testing, PALA was effective against a unique spectrum of solid tumors; Lewis lung carcinoma, B16 melanoma, glioma 26, colon adenocarcinoma 26, and Madison 109 lung carcinoma were inhibited, whereas the drug was inactive against L1210 leukemia and showed only minimal activity in P388 leukemia. PALA caused no myelosuppression, even at overtly lethal doses (11).

A preclinical toxicologic evaluation of PALA was performed utilizing BDF_1 mice, beagle dogs, and rhesus monkeys.[6] The toxic manifestations of the drug in dogs and monkeys were primarily related to gastrointestinal disturbances (such as anorexia, adipsia, weight loss, diarrhea, and hematochezia) and central nervous system effects (characterized by anesthesia or sedation, coma, convulsions, fasciculations, incontinence of urine and stool, pupil-

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[3]Presented in part at the 15th annual meeting of the American Society of Clinical Oncology, New Orleans, LA, May 14-16, 1979.

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[5]We wish to thank Dr. John R. Wellinger for the preparation and interpretation of the histologic sections.

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lary changes, ataxia, dyspnea or apnea, disorientation, weakness, lethargy, and hypothermia). Monkeys were also observed to have peeling or flaking of the skin and alopecia. Only mild and unpatterned alterations of hematopoietic or clinical chemistry parameters were observed at sublethal dose levels.

The schedule of treatments influenced the extent and severity of PALA toxicity. Cumulative toxicity was evident in dogs. A total dose of 300 mg/kg (6000 mg/m²) of PALA when given in five equal injections daily was lethal and a total dose of 480 mg/kg (9600 mg/m²) when given as a single dose produced lethality. When PALA was given as three series of five daily treatments with 9-day rest intervals, a cumulative dose of 450 mg/kg (9000 mg/m²) was well-tolerated. Also, when a total dose given in ten equal daily doses was compared to an equivalent total dose given in only 5 days, the same pattern emerged as when rest intervals were permitted. Monkeys tolerated higher doses of PALA than dogs.

PALA was shown to be a specific inhibitor of ATCase (12). In vivo inhibition of spleen ATCase was maximal 24–72 hours after a single sc injection of 1 mg in the mouse (13). In both in vitro and in vivo systems, uridine could reverse the effects of PALA, and exogenous carbamyl-DL-aspartate, the end-product of the enzymatic reaction, blocked the antitumor activity of PALA, providing strong evidence that the antitumor activity was due to the inhibition of ATCase (14,15). Recently, sensitivity of rodent tumors to PALA has been found to correlate with the level of activity of ATCase, both in vitro (11) and in vivo (16).

Because of PALA's unusual spectrum of antitumor activity in preclinical studies and its unique biochemical mechanism, we initiated a phase I study of the drug in patients with advanced cancer and evaluated the clinical effects of continuous infusions of 24 and 120 hours' duration, repeated every 3 weeks.

PATIENTS AND METHODS

PALA was provided by the National Cancer Institute (Bethesda, MD) in 10-ml vials containing 1 g of PALA in water for USP injection, and NaOH to adjust the pH to 6.5–7.5. Vials were stored at 4°C prior to use, and were prepared for infusion by mixing with an appropriate volume of 5% dextrose in ¼ normal saline. The initial dose used was 500 mg/m² for the 24-hour infusion study, which represented one fifth of the toxic dose low in dogs for single iv bolus. We selected a dose lower than the conventional one-third toxic dose low because when given as a 24-hour infusion, pyrazofurin, another pyrimidine inhibitor, produced orotic aciduria and orotidinuria greater than that after iv bolus of the identical dose (17).

Spaced entry of patients was followed in order to diminish the risk of toxic effects for each subsequent patient. After observing the course of three patients at the initial dose level, subsequent cohorts were entered and evaluated at progressively higher dose levels, guided by a Fibonacci Search Scheme. Additional patients were entered at the dose level that produced moderate but non-dose-limiting toxicity. Doses were repeated at 3-week intervals. After the minimum toxic dose was determined in the 24-hour infusion schedule, a 120-hour infusion schedule was evaluated by dividing that dose over 5 days of continuous iv administration. In the absence of dose-limiting toxicity the dose was escalated in subsequent groups of patients as well as in subsequent courses for the same patient.

Thirty-seven patients with advanced solid neoplasms were entered in the study. There were 16 men and 21 women ranging in age from 24 to 85 years, with a median age of 53 years. All patients had a histologic confirmation of their diagnosis. No known curative treatment remained for these patients, and there was no palliative treatment thought to be of benefit to them. The spectrum of diseases treated is shown in table 1. Ninety-five percent of the patients had had prior chemotherapy and 38% had had prior radiotherapy. Initially, patients with a serum creatinine level of > 1.5 mg/dl, bilirubin level of > 2 mg/dl, SGOT of > 100 milliunits/ml, wbc count of $< 4 \times 10^9$/liter, and platelet count of $< 100 \times 10^9$/liter were excluded from the study. In the latter part of the study, after it was determined that PALA produced no hepatic or renal toxicity, patients with moderate hepatic (three patients) or renal (one patient) insufficiency due to neoplastic involvement were included. Six patients had evident third-space fluid (edema and/or ascites). The performance status of the patients was assessed using the Cancer and Leukemia Group B (CALGB) criteria. Sixty-five percent had a performance status of 0, 1, or 2.

Baseline studies included physical examination, measurement of body weight and height, cbcs, complete automated chemical profiles (SMA-6 plus -12), urinalysis, electrocardiogram, chest roentgenogram, and measurements of tumor lesions. Bone marrow aspiration or biopsy was done when permission was given by the patient. Additional clinical studies were carried out as indicated. Clinical toxic effects were graded using the CALGB toxicity scale as follows: *stomatitis*—1+ = erythema or painless

TABLE 1.—Spectrum of diseases treated with PALA and response to therapy

Disease	No. of patients	No. of patients with— Partial response	Stable disease	Progressive disease	No. of patients not evaluable
Breast carcinoma	8			7	1
Bowel carcinoma (5 colon, 1 duodenum)	6		2	4	
Sarcoma*	5	1	1	3	
Malignant melanoma	3		1	2	
Lung cancer†	3		1	2	
Head and neck cancer	3			3	
Renal carcinoma	3			3	
Ovarian carcinoma	1			1	
Prostatic carcinoma	2			2	
Testicular (embryonal) carcinoma	1			1	
Ependymoma	1			1	
Adenocarcinoma of unknown primary	1			1	
Totals	37	1	5	30	1

*2 chondrosarcomas, 1 each lipomixoma, fibrosarcoma, and leiomyosarcoma.
†1 each squamous cell carcinoma, large cell carcinoma, and alveolar cell carcinoma.

superficial ulcers; 2+ = ulcers, patient can eat; 3+ = ulcers, patient cannot eat; and 4+ = ulcers, infected and/or bleeding; *diarrhea*—1+ = minimal, transient, one to two stools per day for 1–2 days; 2+ = three to six stools per day for > 3 days, controlled with drugs; 3+ = > six stools per day, uncontrolled; and 4+ = gross bleeding and/or dehydration and/or electrolyte imbalance; and *skin*—1+ = transient erythema, macules, and papules; 2+ = vesicles and subepidermal fibrosis; 3+ = ulcerations, necrosis, severe pain, and desquamation; and 4+ = exfoliation.

The study was discontinued for each patient when there was unambiguous disease progression in the presence of toxicity, unacceptable toxicity in the absence of therapeutic effect, or refusal of the patient to continue in the protocol. Therapeutic effects were evaluated using our standard criteria (18).

RESULTS

Twenty-seven of the 37 patients were given PALA as a 24-hour continuous infusion, and received a total of 55 courses. Ten patients were given a 120-hour continuous infusion, and received a total of 20 courses. The dose escalation scheme and toxic effects encountered at each dose level are shown in tables 2 and 3. One patient died on Day 4 with progressive disease and was not evaluable.

Cutaneous and gastrointestinal toxic effects were dose-limiting for both administration schedules, and increased in frequency and severity with increasing dose. In the 24-hour infusion schedule (table 2), 2500 mg/m²/24 hours produced no toxic effects except grade 2+ stomatitis in one patient with ascites, leg edema, and an elevated bilirubin level. An infusion of 4000 mg/m²/24 hours produced mild, transient dermatitis, mucositis, and diarrhea in a small number of patients. Dose escalation to 6500 mg/m²/24 hours resulted in dermatitis in eight of 12 evaluable courses. Because of this, an intermediate dose escalation of 33% (8700 mg/m²/24 hours) was tested. Since no dose-limiting toxicity was seen at this dose level, subsequent patients were evaluated at 10,500 mg/m²/24 hours. This dose was tolerated by one patient during two courses with only mild dermatitis, but appeared to be causally related to the death of a second patient. This patient was a 49-year-old woman (performance status of 3) with breast carcinoma and hypercalcemia (being treated concurrently with hydration, furosemide, phosphosoda, and steroid) who developed moderate dermatitis (grade 2+), severe mucositis (grade 3+), and diarrhea with frank rectal bleeding (grade 4+). By Day 11 after treatment, these toxic manifestations had virtually resolved, but myelosuppression, first noted on Day 5, progressed (wbc count 0.4 × 10^9/liter and platelet count 15 × 10^9/liter). She died on Day 12 of sepsis which originated from a clinically occult chest wall abscess.

Continuous infusion of PALA over 120 hours produced an essentially identical pattern of toxicity (table 3). At an infusion of 8700 mg/m²/120 hours, dermatitis followed every course (three of three),

TABLE 2.—Dose escalation and toxicity (24-hr infusion)

Dose (mg/m^2/24 hrs)	No. of patients (No. of courses)	No. of patients: Toxic effect (severity)				
		Dermatitis	Mucositis	Diarrhea	Leukopenia nadir (cells × 10^9/liter)	Thrombocytopenia nadir (cells × 10^9/liter)
500	3 (3)	0	0	0	0	0
1000	3 (3)	0	0	0	0	0
1650	2 (2)	0	0	0	0	0
2500	5 (5)	0	1 (2+)*,† 1 (1+)*	0	0	0
4000	9 (11)	4 (1+)	1 (2+)	1 (1+)*,‡	0	0
6500	9 (13)§	6 (1+) 1 (2+) 1 (4+)	2 (1+) 1 (2+)	2 (1+)	2 (2.8, 1.5¶)	1 (32)¶
8700	7 (15)	3 (1+) 2 (2+)	4 (1+)	3 (1+) 1 (3+)	1 (2.9)	0
10,500	2 (3)	1 (1+)** 1 (2+)**	1 (3+)**	1 (4+)**	1 (<0.4)**	1 (15)

*Pre-existing edema, ascites, or effusions.
†Pre-existing hepatic dysfunction.
‡Pre-existing renal dysfunction.
§1 course not evaluable bacause of death from progressive disease on Day 4.
¶Received 1000 rads of radiotherapy on Day 6, tumor infiltration of marrow, and wbc count nadir on Day 10.
**Bone marrow infiltration with breast carcinoma.

while two of three courses resulted in mucositis and one course resulted in diarrhea. Although this study was not designed to directly compare the toxicity of 24-hour infusions with 120-hour infusions, the two groups of patients were similar (the mean performance status was 2.1 for the 24-hour infusion group and 1.6 for the 120-hour infusion group; the median performance status was 2.0 for the two groups). Toxicity was encountered less frequently at each dose level in the 24-hour infusion group than in the 120-hour infusion group, suggesting that PALA is more toxic when administered slowly over 5 days (table 4).

The time course of the toxic effects encountered after 24-hour and 120-hour infusions of PALA was similar. The median days of onset and resolution of dermatitis were Days 7 (range, 4–12) and 10 (range, 7–26), respectively. The other toxic effects followed a parallel course. The median days of onset and resolution of mucositis were Days 6 (range, 4–9) and 11 (range, 8–13), respectively, and of diarrhea were Days 8 (range, 5–21) and 11 (range, 10–23), respectively. Skin toxicity was characterized by discrete erythematous macules which developed in intertriginous areas, at pressure points, and over the face. These gradually faded to brown macules and resolved by superficial skin flaking, leaving no residual mark. In instances of more severe reaction, the erythematous macules evolved to vesicles and bullae with subsequent superficial erosions. Skin biopsies were obtained from three patients during different phases of the skin reaction. Initially, prominent vacuolar degeneration of the basal cells were noted (fig 1A). This was followed by sub-basal bulla formation and scattered mononuclear infiltration of the dermis (fig 1B). In another biopsy, regeneration of the basal cells was observed with an accompanying intense inflammatory reaction in the dermis (fig 2). One episode of life-threatening cutaneous toxicity occurred in a patient with breast carcinoma and prominent lymphedema of the left arm who received a 120-hour infusion at 6500 mg/m^2. Erythematous and vesicular eruptions appeared on Day 6 and spread to involve 50% of the body surface area by Day 11, with desquamation involving approximately 30% of the skin area. Large quantities of balanced electrolyte solution were required to restore fluid balance and correct prerenal azotemia. The cutaneous reaction resolved by Day 30.

Gastrointestinal toxicity included mucositis and diarrhea. Instances of mild mucositis were characterized by circumoral burning sensations, oral pain, and cheliosis. In several patients, this progressed to superficial ulceration of the oral mucosa which limited food and fluid intake. The mucositis resolved in all patients within 2 weeks.

Diarrhea was usually mild, easily controlled, and not associated with occult bleeding. In two instances the diarrhea was severe, accompanied by gross bleeding and a hemoglobin drop of > 1 g/dl. In one patient requiring transfusions of 3 units of

blood, hyperemia with multiple erosions of the colonic mucosa was documented.

Fourteen patients received multiple courses of PALA (mean, 3.6 courses; range, two to seven) at doses of 4000–10,500 mg/m²/24 hours and 4000–8700 mg/m²/120 hours. There was no evidence of cumulative toxicity.

To identify factors that might predict toxicity from PALA we looked for common characteristics among patients who had toxic reactions. Patients with third-space fluid compartments appear to have developed more frequent and more severe toxic reactions. For example, at the dose level of 4000 mg/m²/24 hours, no toxic manifestations developed in six patients without third-space fluid, while all three patients with ascites or edema developed toxic effects: dermatitis (three of three patients), mucositis (two of three), and diarrhea (one of three). Only one of the three patients with impaired hepatic function (bilirubin levels of 2.8, 5.8, and 12.5 mg/dl) developed any toxic effect (grade 2+ mucositis) and this patient also had ascites and peripheral edema. The poor performance status of the patients with third-space fluid (mean, 2.8 compared with 1.9 for the total study population) may also have contributed to their toxicity.

Moderate myelosuppression was seen in patients with histologically proven neoplastic infiltration of bone marrow. Among the remainder of patients, myelosuppression occurred only twice, was less severe, and was associated with prominent cutaneous and gastrointestinal toxicity. No drug-related hepatic, renal, cardiac, or neurologic toxicity was noted. Inadvertent paravenous infiltration of the dilute solutions of PALA resulted in only mild transient erythema and tenderness.

Six of the 36 evaluable patients showed some evidence of antitumor activity of PALA (table 1). A partial response was seen in a previously untreated 53-year-old woman with a large (28 × 21-cm) gluteal chondrosarcoma arising from a sacral vertebra. After a course of 2500 mg/m²/24 hours, a minor reduction in the gluteal mass was noted. After a subsequent course of 6500 g/m²/24 hours, the tumors

TABLE 3.—Dose escalation and toxicity (120-hr infusion)

Dose (mg/m²/120 hrs)	No. of patients (No. of courses)	No. of patients: Toxic effect (severity)				
		Dermatitis	Mucositis	Diarrhea	Leukopenia nadir (cells × 10^9/liter)	Thrombocytopenia nadir (cells × 10^9/liter)
4000	4 (4)	1 (1+) 1 (4+)*	1 (2+) 1 (4+)*	2 (1+)* 1 (2+) 1 (4+)	1 (3.2)*	0
6500	8 (13)	5 (1+) 2 (2+) 1 (4+)†	3 (1+) 1 (2+) 4 (3+)†	1 (2+) 1 (4+)†	3 (3.4†, 2.8‡, 1.9‡)	1 (59)‡
8700	2 (3)	1 (1+) 1 (2+) 1 (3+)	2 (1+)	1 (1+)	0	0

*Ascites and pleural effusion.
†Prominent lymphedema of the arm.
‡Bone marrow infiltrated with prostatic carcinoma.

TABLE 4.—Comparison of toxic effects of 1st course of PALA by 24- and 120-hr infusions

Total dose (mg/m²)	No. of patients with toxic effects/total No. of patients (%)			
	Dermatitis	Mucositis	Diarrhea	Leukopenia
4000/24 hrs	3/9 (33)	2/9 (22)	1/9 (11)	0/9 (0)
4000/120 hrs	2/4 (50)	2/4 (50)	3/4 (75)	1/4 (25)
6500/24 hrs	5/9 (55)	1/9 (11)	0/9 (0)	0/9 (0)
6500/120 hrs	5/8 (62)	4/8 (50)	2/8 (25)	2/8 (25)
8700/24 hrs	4/7 (57)	3/7 (43)	3/7 (43)	0/2 (0)
8700/120 hrs	2/2 (100)	2/2 (100)	1/2 (50)	0/2 (0)

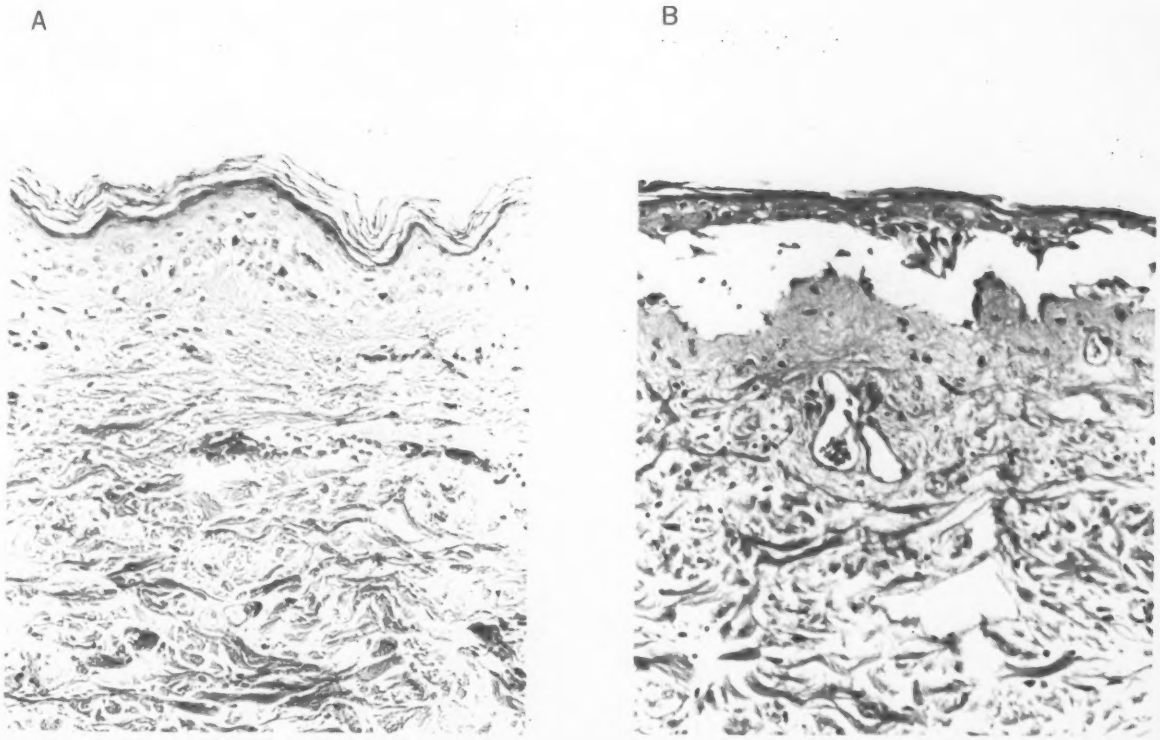

FIGURE 1.—Skin biopsies. A. 9 days after a dose of 8700 mg/m²/24 hrs of PALA, showing prominent vacuolar degeneration of basal cells. B. 12 days after a dose of 10,500 mg/m²/24 hrs of PALA, showing sub-basal bulla formation. Scattered mononuclear infiltrates are present in the dermis (H and E stain, × 100).

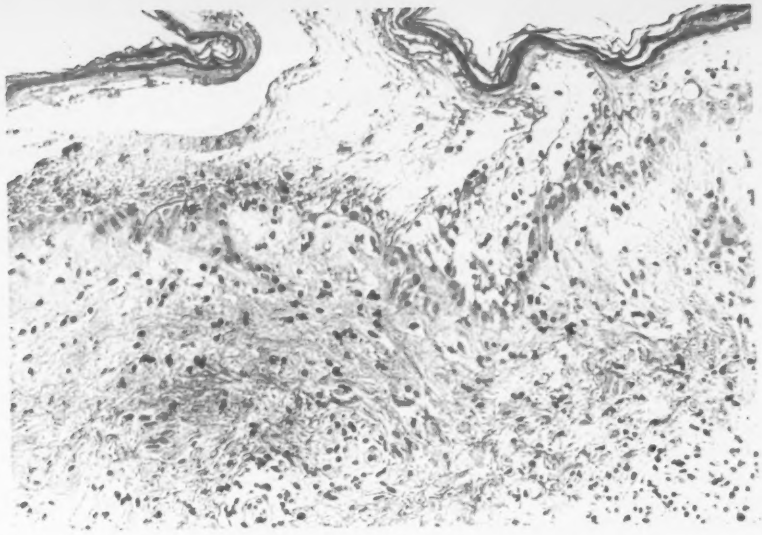

FIGURE 2.—Skin biopsy, 6 days after a dose of 6500 mg/m²/24 hrs of PALA, showing sub-basal bullae with regeneration of basal cells. Intense inflammatory reaction is seen in the dermis (H and E stain, × 100).

decreased to 16 × 17 cm. She was then given 8700 mg/m²/24 hours every 3 weeks for three courses, with no further change in the size of the tumor. Five months after starting PALA she was noted to have progressive disease necessitating a change to other drugs. A minimal antitumor effect of PALA was seen in five patients whose measurable tumor decreased by < 25% or stopped increasing in size for a period of 2–6 months (table 1).

DISCUSSION

The present study establishes the parameters of toxicity of PALA given as a 24- or 120-hour infusion in man. The dose-limiting toxic effects occurred in the skin and gastrointestinal tract. Clinically important hematopoietic toxic effects occurred only in those patients with neoplastic marrow involvement. Neurologic toxic effects observed in large-animal toxicologic studies were not recognized in man. Available data indicate that the recommended dose for phase II study is 8700 mg/m²/24-hour infusion every 3 weeks or 6500 mg/m²/120-hour infusion every 3 weeks in patients with no major organ dysfunction and no third-fluid space. Pre-existing hepatic dysfunction did not seem to influence severity of toxicity. Relationship between pre-existing renal dysfunction and severity of toxicity could not be established, but since 50%–80% of administered PALA is excreted in the urine unchanged (Ohnuma T, unpublished data), it is suggested that patients with renal dysfunction should receive PALA at a reduced dose. Repeated courses of treatment did not produce cumulative toxicity.

The reasons for the severe toxic effects encountered in one of two patients who received 10,500 mg/m²/24 hours are unknown. The patient had a normal serum creatinine and no increase in third-space fluid. However, while she was receiving PALA, she was also being treated with hydration, furosemide, phosphosoda, and steroid for hypercalcemia secondary to bone metastasis. It is possible that the concomitantly administered phosphosoda competed with PALA for renal excretion.

In conjunction with the overall strategy of the National Cancer Institute to assure that different dose schedules of phase I compounds are studied, we explored the toxicity of PALA given by infusion. Earlier experience with other antineoplastic agents suggested to us that administration by infusion produced an increase in therapeutic effects (19,20) or a different spectrum of toxic effects when compared to iv bolus (18). Infusional treatments may also have the advantage that the effects of the serum drug concentration achieved in man can be tested in in vitro systems.

The 120-hour infusion schedule in this study produced a pattern of toxicity identical to the 24-hour schedule, but with an increase in the severity at any one dose level. This suggests that the length of exposure is important for the development of toxicity and is consistent with preclinical data showing that PALA was rapidly cleared from the plasma and excreted in the urine (21). This may also explain the increased severity of toxic effects seen in patients with third-space fluid compartments that may act as a reservoir from which the drug is slowly released. Infusions of PALA may have an advantage over iv bolus administration in that the acute paresthesias observed after iv bolus of PALA (22) have not been seen in this study.

We observed one partial response lasting 5 months in a patient with chondrosarcoma. Since PALA is deposited and retained in mouse bone (21), study of the antitumor activity in other patients with chondrosarcoma or osteosarcoma may be of value. In addition, a patient with a large leiomyosarcoma of the abdomen had disappearance of leg edema, improvement in well-being, and slight reduction in the size of an abdominal tumor for 6 months. Four other patients in this study had stabilization of their disease for 2–6 months and clinically appeared to have benefited from PALA.

PALA, one of a new class of antineoplastic agents, has exhibited tolerable, predictable, reversible toxicity. Lack of dose-limiting hematologic toxicity suggests a role of PALA in combination with myelosuppressive agents.

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Phase II Evaluation of Diglycoaldehyde (Inox) in Children With Acute Leukemia: A Children's Cancer Study Group Report[1]

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SUMMARY

A phase I trial of diglycoaldehyde (Inox) in childen with leukemia established that the maximum tolerated dose of a 5-day schedule was 1.5 g/m²/day. A phase II study was undertaken by the Children's Cancer Study Group to evaluate the efficacy of this dose. Forty-seven children with acute leukemia refractory to conventional forms of therapy were entered in the study: 29 patients with acute lymphocytic leukemia/acute undifferentiated leukemia and 18 with acute nonlymphocytic leukemia. Inox was administered at a dose of 1.5 g/m² as a 4–6-hour iv infusion daily for 5 days every 14 days. Toxic effects included myelosuppression, proteinuria, nausea, vomiting, diarrhea, local tissue reactions, hypocalcemia, transitory serum amylase elevation, and transitory hypotension. There was one life-threatening episode of drug-related renal toxicity. Of the 27 patients who received a minimum of two courses, partial remissions were observed in two patients with acute nonlymphocytic leukemia. Inox was inactive against advanced acute lymphocytic leukemia.

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[2]This study was conducted by the Children's Cancer Study Group. The following institutions (with investigators and grant support) participated in the study: University of Southern California Comprehensive Cancer Center, Los Angeles (*D. Hammond, J. Weiner, R. Honour, H. Sather,* and *J. Anderson,* CA-13539); University of Michigan Medical Center, Ann Arbor (*R. Heyn,* CA-02971); Children's Hospital National Medical Center and George Washington University, Washington, DC (*S. Leikin,* CA-03888); Memorial Sloan-Kettering Cancer Center-Cornell University, New York, NY (*D. R. Miller,* CA-14557); Children's Hospital of Los Angeles and University of Southern California (*G. Higgins,* CA-02649); Babies Hospital and Columbia University, New York, NY (*J. Wolff,* CA-03526); Children's Hospital of Pittsburgh and University of Pittsburgh, PA (*V. Albo,* CA-07439); Children's Hospital of Columbus and Ohio State University (*W. Newton,* CA-03750); Childen's Orthopedic Hospital and Medical Center and University of Washington, Seattle (*R. Chard,* CA-10382); University of Wisconsin Hospitals, Madison (*N. Shahidi,* CA-05436); University of Minnesota Health Sciences Center, Minneapolis (*M. Nesbit,* CA-07306); Children's Memorial Hospital and Northwestern University, Chicago, IL (*G. Honig,* CA-07431); University of Utah Medical Center, Salt Lake City (*E. Lahey,* CA-10198); Princess Margaret Hospital and University of Toronto, Ontario, Canada (*M. Sonley,* Ontario Cancer Treatment and Research Foundation grant 41-17); Strong Memorial Hospital and University of Rochester, NY (*M. Klemperer,* CA-11174); Children's Hospital and University of Louisville, KY (*D. R. Kmetz*); University of British Columbia, Vancouver, Canada (*M. Teasdale,* Vancouver Foundation); Children's Hospital of Philadelphia and University of Pennsylvania (*A. Evans,* CA-11796); James Whitcomb Riley Hospital for Children and Indiana University, Indianapolis (*R. Baehner,* CA-13809); New Jersey College of Medicine and Dentistry, Newark (*L. Vitale,* CA-12637); Harbor General Hospital-University of California at Los Angeles, Torrance (*J. Finklestein,* CA-14560); University of California Medical Center, San Francisco (*A. Ablin,* CA-17829); and Rainbow Babies and Children's Hospital and Case Western Reserve University, Cleveland, OH (*S. Gross,* CA-20320). All CA grants are from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

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Diglycoaldehyde (Inox) is the periodate oxidation product of the purine nucleoside inosine in which carbons 2′ and 3′ of the riboside ring have been oxidized to formyl groups with resultant cleavage of the ring (fig 1).[4] The mechanism of action of Inox has been speculated as involving a Schiff's type reaction of the two aldehyde groups (1,2). The drug inhibits RNA and DNA synthesis (3,4). One of the major sites of action is inhibition of ribonucleotide reductase (5,6). Inox is active in several animal tumor systems including L1210, P388, and AKR murine leukemias, and it is marginally active against Lewis lung carcinoma.[4] Studies in laboratory animals indicate that the drug binds avidly to plasma proteins and is excreted in the urine (7). In monkeys and beagle dogs, Inox produced hypotension accompanied by bradycardia, glomerular and tubular lesions in the kidneys, marked azotemia, and minimal myelotoxicity (8).[4]

In initial clinical trials in adults, the dose-limiting toxic effect was renal tubular damage with reversible acute renal failure (8,9). A phase I evaluation of Inox was performed in children by investigators associated with Children's Cancer Study Group (CCSG).[5] A dose of 1.5 g/m² iv daily for 5 days produced manageable toxicity in patients with acute leukemia. On the basis of adult and pediatric phase I data, a group-wide phase II study in children was begun by the CCSG in December 1975.

MATERIALS AND METHODS

Children with acute leukemia refractory to standard methods of therapy were eligible for this study. Eligibility requirements included relapse confirmed by marrow examination and no chemotherapy or radiotherapy for 2 weeks prior to entry in this study. Informed consent was required from the patient, parent, or guardian prior to entry in the study. Pretreatment evaluation requirements included history and physical examination, cbc, bone marrow examination, serum creatinine, BUN, serum uric acid, liver function tests, serum calcium, serum phosphorus, serum amylase, direct Coombs' test, and urinalysis. Prior to each course, observations included physical examination, cbc, serum electrolytes, serum creatinine, BUN, serum calcium, liver function tests, serum amylase, and urinalysis. On Days 3 and 5 of each course, cbc, serum calcium, serum creatinine, BUN, serum electrolytes, and urinalysis were required. A bone marrow examination was required on Day 28 and, if rated M_2, repeated on Day 56.

Response to therapy was evaluated according to criteria previously published by the CCSG (10,11).

Inox was supplied as a lyophilized powder in 1-g vials by the Drug Evaluation Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). Each vial of the drug was reconstituted with 9 ml of sterile water and rediluted with 300 ml of 5% dextrose in water containing 15 mEq of sodium bicarbonate to adjust the pH to 7.4. Inox was administered by iv infusion at a dose of 1.5 g/m² over 4–6 hours daily for 5 days. Courses were repeated every 14 days provided recovery from toxic side effects had occurred. Therapy was discontinued at any time due to the development of unacceptable drug toxicity. For patients with an absolute neutrophil count of $< 500/mm^3$ and no blast cells in the peripheral blood, a bone marrow examination was made. Full drug dosage was given if the marrow rating was M_3; 50% dosage was given if the rating was M_2; and the course was to be delayed until the neutrophil count was $> 1000/mm^3$ if the rating was M_1. A minimum of two courses constituted an adequate trial. Treatment was discontinued in any patient who failed to show a therapeutic response following four courses of therapy.

RESULTS

Forty-seven children with advanced acute leukemia were entered in the study. There were 27 males and 20 females ranging in age from 2 to 20 years. Twenty-nine patients had acute lymphocytic leukemia/acute undifferentiated leukemia (ALL/AUL) and 18 had acute nonlymphocytic leukemia (ANLL).

Twenty of the 47 patients entered had inadequate trials. Of these 20, four were due to parental refusal to continue therapy, six were due to protocol error, and ten died within 3 weeks of entry in the study (table 1). Twenty-eight patients had a minimum of two courses of Inox; of these, one had three courses and two had four courses. No therapeutic responses were observed in 18 evaluable patients with ALL/AUL. Partial remissions were observed in two of nine patients with ANLL. One patient with acute myelogenous leukemia achieved an M_2A marrow (12% blast cells) after two courses of Inox and had an M_2B marrow (27.5% blast cells) 14 days later following an additional course of drug at one-half dose because of neutropenia. A second patient with

[4]Helman L, and Slavik M. Clinical brochure: diglycoaldehyde (NSC-118994). Prepared by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, July 1976.

[5]Handelsman H, Legha S, Penta J, et al. Diglycoaldehyde. *In* Minutes of the Phase I Working Group Meeting. Bethesda, MD, National Cancer Institute, Feb 1975, pp 29–36.

TABLE 1.—Diagnosis and response of children receiving Inox

Diagnosis	No. of patients	No. of patients receiving ≥ 2 courses	No. of responses			
			Complete remission	Partial remission	No response	Inadequate trial
ALL/AUL	29	18	0	0	18	11
ANLL	18	9	0	2	7	9
Totals	47	27	0	2	25	20

acute myelogenous leukemia had an M_2A marrow (12% blast cells) on Day 28 after two courses of Inox. Following two additional courses of Inox, the patient had an M_3 (100% blast cells) marrow on Day 56.

Forty-five of the 47 patients were evaluable for toxicity (table 2), since two patients died of sepsis within 3 days. The incidence of recorded toxicity was low. Hematologic toxicity was recorded in four patients. A reduction in drug dosage was required in only the one patient with an M_2A marrow and an absolute neutrophil count of $< 500/mm^3$ on Day 34 prior to a third course of Inox.

There was one life-threatening episode of drug-related renal toxicity. A patient with ANLL developed *Pseudomonas* sepsis during the first course of Inox. Treatment with gentamicin and carbenicillin was begun. After the second dose of Inox during the second course of therapy, the patient developed an elevated BUN (36 mg/dl) and creatinine (2.8 mg/dl). Inox was discontinued and the patient died 9 days later with progressive renal failure. Renal toxic effects were observed in three additional patients and consisted of proteinuria: 200–600 mg/dl in two patients and 8.5 g/24-hour urine in one patient. One of the three patients had transitory elevation of the BUN to a maximum value of 63 mg/dl.

A serum calcium of < 9 mg/dl occurred by Days 3–5 of the first course in 11 of 17 patients who were monitored with repeated serum calcium determinations, and by Days 1–8 of the second course in three patients. There were no reports of tetany. Calcium gluconate was administered iv in two patients in order to maintain a serum calcium level of 9 g/dl. Transitory elevations in serum amylase were observed in one patient. Gastrointestinal toxic effects observed in nine of 44 patients included nausea, vomiting, and diarrhea. Although no neurotoxicity was recorded, one patient with ALL developed weakness of the left arm and leg while receiving Inox, and it was noted that his neurologic condition improved after Inox was discontinued. That patient had a history of focal motor seizures secondary to CNS chemotherapy, which was being controlled by treatment with diphenylhydantoin. There were four local tissue reactions consisting of pain and/or rash or blisters at the injection site. One patient with ALL experienced transitory hypotension following drug administration. There were no documented instances of hemolysis. The Coombs' test was not reported consistently; however, five of six patients converted to a positive Coombs' after two doses of Inox.

DISCUSSION

The purpose of the study was to determine the efficacy of Inox in acute leukemia of childhood. A dose of 1.5 g/m^2 daily for 5 days every 2 weeks was

TABLE 2.—Toxicity of Inox in children

Toxic effect	No. of patients with toxicity grade*—					Total
	0	1	2	3	4	
Hematologic	41	2	2	0	0	45
Renal	40	1	3	0	1	45
Gastrointestinal	36	5	3	1	0	45
Hepatic	45	0	0	0	0	45
Neurologic	45	0	0	0	0	45
Local skin	41	1	3	0	0	45

*Grade 0 = none; 1 = mild; 2 = moderate; 3 = severe; 4 = life-threatening. Specific limits for each toxicity grade depend on the organ system and the patient's age. (CCSG toxicity guidelines. Available on request from the Operations Office, 1721 Griffin Ave, Los Angeles, CA 90031.)

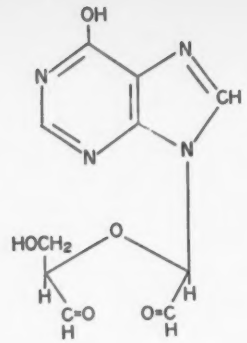

FIGURE 1.—Structure of Inox.

chosen for CCSG group-wide evaluation following the initial clinical trial in 24 children by investigators associated with CCSG. This dose and schedule produced reversible renal toxic effects in children with advanced acute leukemia. The choice of dose was supported by concurrent studies in adults in whom renal toxic effects were dose-limiting at 2 g/m² daily for 5 days given monthly (8). A dose of 2 g/m² daily for 3 days every 28 days was recommended for phase II trials in adults (9). Other dose-related toxic effects observed during the phase I pediatric evaluation included pain at the site of infusion, nausea and vomiting during infusion, hypocalcemia, elevated amylase, and phlebitis (*see* footnote 5). Supplemental calcium was routinely given to patients in the latter part of the phase I study. Following the experience of Kaufman and Mittelman (9), phlebitis was avoided after the drug was buffered with sodium bicarbonate and the infusion administered over 4 hours. A positive Coombs' test was observed during the phase I study; however, none of the children had clinical or laboratory evidence of hemolysis.

In the phase II study, the incidence of recorded toxicity was low. This was partly due to the absence of clinical signs of hypocalcemia and the reduction of phlebitis by buffering the drug. One patient with *Pseudomonas* sepsis died 9 days after Inox therapy with renal failure. No other significant toxicity was encountered.

The 20 inadequate trials in this study reflect the advanced disease in this group of patients. Because Inox was discontinued in six patients when no response occurred after one course of therapy and there were ten early deaths due to progression of disease, the study of Inox was prolonged in order to complete the objective of the study.

Since the dose and schedule of Inox used in this study were the same or greater than doses used in other trials of this agent, it is apparent that Inox has little if any therapeutic value as a single agent in the treatment of advanced ALL/AUL. It has weak activity in ANLL, and the rate of response is probably not frequent enough to warrant further clinical trials.

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Distribution and Inhibition of Adenosine Deaminase in Tissues of Man, Rat, and Mouse[1,2]

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SUMMARY

Adenosine deaminase activity, with arabinosyladenine (ara-A) used as the substrate, was measured in human leukemic cells and major tissues of man, rat, and mouse. The enzyme is stable in frozen tissues for at least 1 week, in frozen homogenate for several months, or after overnight dialysis at 4°C in 0.25 M sucrose. The adenosine deaminase of chronic myelogenous leukemic (CML) cells has a K_m of 0.6 mM and a V_{max} of 183 μmols × 10^{-2}/g of cells/minute. All species have highest activities in spleen and intestine. Kidney activity is highest in rat and lowest in man. The human brain has higher activity than the mouse or rat brain, and activity is higher in mouse liver than in human or rat liver. Activity in lung tissues is moderate and similar in all three species. The mean activity in CML cells is 195 μmols × 10^{-2}/g of cells/minute, and the activity is higher in patients with CML in blast cell crisis. Acute myelogenous and lymphocytic leukemia cells also have very high activities. Drug concentrations required for inhibiting 50% of CML enzyme activity are 10 nM of erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), 0.6 nM of coformycin, and 0.3 nM of 2′-deoxycoformycin. Preincubation of the enzyme with coformycin, but not with EHNA, enhances the inhibition. In view of the high adenosine deaminase activities in tissues and the enhancement of the therapeutic effect of ara-A by inhibitors in mouse tumor systems, clinical trials of these combinations are warranted. They may be particularly beneficial to patients with CML in blast cell crisis.

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9-β-D-Arabinosyladenine (ara-A) was first synthesized as a potential anticancer agent in 1960 (1). Ara-A has antiviral effects in experimental systems and in man (2) and is clinically effective in treating patients with chronic myelogenous leukemia (CML) (3). In man and animals ara-A is rapidly deaminated by adenosine deaminase (EC 3.5.4.4) to arabinosylhypoxanthine (ara-H), which is apparently not active against experimental tumors known to be sensitive to ara-A (4). Mouse tumors with relatively high adenosine deaminase activity are unresponsive to ara-A, while tumors with low deaminase activity are sensitive to the drug (5). Several potent inhibitors of adenosine deaminase, 2′-deoxycoformycin (dCOF), coformycin (COF), and erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), have been isolated or synthesized (6–8). Using ara-A as the substrate, the deaminase activities were measured in major tissues of man, rat, and mouse and in human leukemic cells. The effects of the three inhibitors on the adenosine deaminase activity were compared in CML cells.

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MATERIALS AND METHODS

Ara-A, ara-A-[adenine-2-^{3}H(*N*)] (specific activity, 11 curies/mmol), and ara-H were supplied by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). The radiochemical purity was > 97% as determined by chromatographic and radiochemical techniques. COF, dCOF, and EHNA were gifts of Dr. H. Umezawa, Microbial Chemistry Research Foundation (Tokyo, Japan), Dr. A. J. Glazko, Parke-Davis and Co (Detroit, MI), and Dr. H. J. Schaeffer, Virginia Medical College (Richmond, VA), respectively. Phase-combining solvent (PCS) was purchased from Amersham/Searle Corp (Arlington Heights, IL). Other chemicals were commercially available.

Male DBA/2 and BDF_1 mice (20–25 g) and male Sprague-Dawley rats (350–400 g) were purchased from Texas Inbred Mouse Co (Houston, TX). Animals were killed by cervical dislocation and tissues were dissected immediately. Human autopsy tissues were obtained within 4 hours of traumatic death. Human leukemic cells were collected from an IBM blood cell separator (9). After rbcs were removed by hypotonic shock (10), the leukemic cells were sonicated with 4 vol of 0.25 M sucrose. The homogenate was centrifuged in a Beckman ultracentrifuge (Model L2-65B) at 180,000 × *g* for 30 minutes.

Tissues were homogenized with 3 vol of 0.25 M sucrose, and the homogenate was centrifuged at 180,000 × *g* for 30 minutes. The centrifuged cell and tissue supernatants were frozen at –10°C until assay. The enzyme was stable for several months in the frozen state. With the exception of mouse intestine, the enzyme activity of the supernatant centrifuged for 30 minutes at 180,000 × *g* was comparable to that of supernatant centrifuged for 30 minutes at 25,000 × *g*. The preparation of 180,000 × *g* was routinely used. All of the above procedures were carried out at 4°C unless otherwise specified.

Enzyme Assay

The adenosine deaminase assay was performed in triplicate. Tris buffer (pH 8.0, 30 μmols), ara-A (0.3 μmols; 0.34 microcurie of tritiated compound), and an appropriate amount of the enzyme preparation in a total volume of 0.3 ml were used. The enzyme reaction was performed at 37°C for 5–60 minutes and was terminated by heating for 5 minutes in a boiling water bath. The enzyme reaction was linear for at least 2 hours. Boiling or using perchloric acid to remove proteins gave comparable results, and for convenience, the boiling method was routinely used. After centrifuging in a International Clinical Centrifuge (Model CL) for 5 minutes, an aliquot was applied on Whatman No. 1 paper with appropriate markers. The chromatograms were developed descendingly for 6–7 hours in several solvent systems, and the R_f values of ara-A and ara-H from each system are shown in table 1. System 3 was chosen for routine use and the time was lengthened to overnight to allow better separation. No adenine or evidence of tritium exchange was found in the reaction mixture. The fluorescent spots were identified with an ultraviolet light and cut from the paper. One milliliter of H_2O was added to each vial and allowed to stand for 10 minutes. Ten milliliters of 66% PCS in xylene was then added and counting was done in a Packard Liquid Scintillation Spectrometer (Model 3375). The counting efficiency for ^{3}H was 37%, and quenching was corrected by external standardization.

The enzyme had a broad optimum pH which ranged from 7.0 to 8.5. Comparable enzyme activities were obtained when the homogenizing medium was one of the following: H_2O; 0.05 M Tris buffer (pH 8.0); 0.25 M sucrose; or 0.25 M sucrose containing 0.05 M Tris buffer (pH 8.0), 0.025 M KCl, 0.005 M $MgCl_2$, and 0.001 M 2-mercaptoethanol. For regular use, 0.25 M sucrose was chosen as a homogenizing medium. Dialyzing homogenate in 0.25 M sucrose at 4°C for 18 hours did not affect the enzyme activity.

The stability of the enzyme was tested in frozen tissues or cells and in fresh tissues. No enzyme activity was lost after the tissues or cells were kept frozen at –10°C for 1 week. The enzyme was also stable in the fresh tissues that were kept at room temperature for 2–3 hours, as well as those placed in an ice bath for an additional 4 hours. These results eliminated the question of any possible loss of activity in human tissues obtained from autopsy several hours after death.

RESULTS

K_m determination.—With ara-A as the substrate, the K_m value for CML cells calculated from the Lineweaver-Burk plot is $5.99 \pm 0.50 \times 10^{-4}$ M and

TABLE 1.—R_f values of ara-A and ara-H in various solvent systems

Solvent systems	R_f	
	Ara-A	Ara-H
5% Na_2HPO_4 (wt/vol)	0.47	0.74
93.8% butanol:44% propionic acid, 50:50 (vol/vol)	0.50	0.34
Ethylacetate:isopropanol:H_2O, 65:23:12 (vol/vol/vol)	0.29	0.19

the V_{max} value is 183 ± 18 μmols × 10^{-2}/g/minute. These values were averages from three determinations ± SE.

Distribution of adenosine deaminase.—The activity was determined in various tissues of man, mouse, and rat (table 2). With the exception of mouse intestine, the enzyme activity was similar in the homogenate or the supernatants obtained after centrifuging the homogenate for 30 minutes at 25,000 or 180,000 × *g*. After centrifuging the homogenate at 180,000 × *g*, the activity in the mouse intestine decreased about 20%, from 290 to 235 μmols × 10^{-2}/g/minute.

Adenosine deaminase activity in human leukemic cells.—After rbcs were removed, the enzyme activity was measured in human leukemic cells from patients with acute myelogenous leukemia (AML), CML, acute lymphocytic leukemia (ALL), or chronic lymphocytic leukemia (CLL) (table 3). The values for four patients who had CML in blast cell crisis are also reported (table 3). The activity was high in patients with CML in blast cell crisis, with AML, and with ALL.

Effect of inhibitors on adenosine deaminase activity.—Various concentrations of EHNA, COF, and dCOF were tested for their inhibitory effects on the CML enzyme activity. The concentrations inhibiting 50% of the enzyme activity (ID50) were calculated to be 10, 0.6, and 0.3 nM for EHNA, COF, and dCOF, respectively. With enzymes prepared from AML or CLL cells, the ID50 values were similar to those obtained with enzymes prepared from CML cells.

Pre-incubating EHNA with the enzyme up to 60 minutes produced the same inhibitory effect as that without pre-incubation. However, pre-incubation of COF with the enzyme for 30 minutes increased its inhibitory effect from 30% to 63%. Dialyzing homogenates in 2 liters of 0.25 M sucrose at 4°C for 18 hours did not increase the enzyme activity or affect the potency of the inhibitors.

DISCUSSION

Adenosine deaminase is widely distributed in tissues of man and rodents, and variation in activities is observed among different species. Compared to adenosine (K_m 4–5 × 10^{-5} M), ara-A (K_m 6 × 10^{-4} M) is not as good a substrate (11,12). Human spleen and small intestine have high adenosine deaminase activities, which, based on the total enzyme activity per organ weight, would result in complete deamination of ara-A if patients were continuously infused with ara-A at a therapeutic dose of 1000 mg/m²/24 hours. This corresponds with the findings of initially high ara-H levels in the plasma and urine of patients after they have received ara-A (13). Bone marrow has relatively lower activity, which may be one of the reasons for myelosuppression in patients treated with ara-A (3). The high adenosine deaminase activity in intestine precludes the possible use of orally administered ara-A.

The plasma adenosine deaminase activity in cancer patients with liver involvement was found to be 1.6- to 4-fold higher than that of normal subjects (14). Since a majority of cancer diseases have liver involvement, it is important to compare clinical pharmacology of ara-A in patients with normal and abnormal hepatic function. If differences exist between the two groups, the therapeutic dose of ara-A may need adjusting.

Adenosine deaminase is known to be inhibited by COF, dCOF, and EHNA (6–8,15), and our work provides evidence that when ara-A is used as the

TABLE 2.—Adenosine deaminase activity in human and rodent tissues (values were obtained from pooled tissues of 10 mice or 4 rats)

	Deaminase activity (μmol × 10^{-2}/g/min)					
	Man			Mouse		
Tissues	No. of studies	Average	Range	BDF_1	DBA/2	Rat
Brain	4	14.3	8.3–21.6	1.7	2.3	4.8
Liver	5	13.0	8.3–15.0	41.0	39.3	15.3
Spleen	5	116.8	113–123	86.8	90.5	97.8
Intestine*	2	64.1	50–78	293.8	298.8	89.7
Kidneys	6	4.5	3.3–5.0	21.7	24.7	58.2
Lungs	4	26.3	21.7–38.3	30.7	38.2	41.0
Rbcs	4	13.5	10–21.7	†	24.8	0.5
Bone marrow‡	2	6.3	6.2–6.4	†	17.5	12.3
Colon	2	22.5	21.7–23.3	†	†	†

*Enzyme activity was determined in the small intestine of man and in the whole intestine of rats.
†Not determined.
‡μmol × 10^{-2}/10^9 cells/min.

TABLE 3.—Adenosine deaminase activity in human leukemic cells

Cells	% blast cells	No. of studies	Deaminase activity (μmol × 10^{-2}/g/min)	
			Mean ± SE	Range
AML	80–90	12	683 ± 170	157–2570
CML	1–6	11	195 ± 15	90–270
CML	Blast crisis	4	618 ± 105	508–828
ALL	95	2	805	668–940
CLL	~100 lymphocytes	2	112	107–118
Normal	—	4	100 ±8	88–108

substrate, the deaminase activity in various human leukemic cells is also inhibited by these agents. The adenosine deaminase activity is also low in CML and CLL cells (16,17). If the correlation of low deaminase activity and sensitivity to ara-A in experimental tumors exists in human systems, the relatively low adenosine deaminase activity in CML and CLL cells may explain the therapeutic effects of ara-A observed in CML patients (3) and may also warrant clinical trials in CLL patients. The concentrations inhibiting 50% of the enzyme activity of CML cells (ID50) are 10, 0.6, and 0.3 nM for EHNA, COF, and dCOF, respectively. Our preliminary experiments indicate that incubation of ara-A with CML cells in the presence of dCOF or EHNA not only inhibits the ara-A deamination, but also increases the ara-A nucleotide formation. In addition, dCOF has been shown to increase ara-A triphosphate (ara-ATP) levels in experimental tumor cells. This nucleotide is the potent inhibitor of DNA polymerase (18,19), and the increase in ara-ATP enhances the inhibition of DNA synthesis. This supports the findings that dCOF increases cytotoxic effects of ara-A in vitro (20) and in vivo (21–23). In the presence of dCOF, ara-A became more active against L1210 leukemia, the tumor innately resistant to ara-A. In view of these results in experimental systems, clinical trials of ara-A in combination with adenosine deaminase inhibitors are warranted in patients with CML in blast cell crisis, with AML, or with ALL. dCOF seems to be the inhibitor of choice for the combination study; however, differences in uptake, biologic half-life, excretion, metabolism, and other factors of these inhibitors should also be considered. For example, the plasma half-life of dCOF is known to be short in animals (24–26). The uptake and biologic half-life of other adenosine deaminase inhibitors in plasma and tissues should be compared with dCOF. Our studies have indicated that the inhibition of ara-A deamination in vitro is enhanced by pre-incubating the enzyme with COF, but not with EHNA (table 2), and a similar phenomenon occurs in vivo (27). To achieve maximum efficacy of the inhibitor in mice, dCOF, but not EHNA, must be given 15 minutes before ara-A. Furthermore, COF and dCOF were recently shown to be potent inhibitors of rabbit muscle 5′-adenosine monophosphate deaminase (5′-AMP deaminase) (EC 3.5.4.6.), but EHNA did not inhibit the enzyme even at the relatively high concentrations of 1–2 × 10^{-4} M (28). Currently, the physiologic function of this enzyme is still under investigation. However, 5′-AMP deaminase is known to be important in regulating the purine nucleotide concentrations which are involved with amino acid metabolism as inhibitors or activators depending on different organs and species (29); inhibition of this enzyme may thus result in toxicity. COF is approximately a 200-fold stronger inhibitor of 5′-AMP deaminase than dCOF, and greatest toxicity with COF than with dCOF and least toxicity with EHNA are predicted. On the other hand, infusion of EHNA in mice for 5 days was shown to increase adenosine deaminase activity in the lungs, stomach, liver, and jejunum (30). This effect was compared with the weaker deaminase inhibitor, 9-β-D-arabinofuranosyl-6-hydroxy-1-amino purine, but not with an equally potent inhibitor such as COF or dCOF. Thus, a particular inhibitor in combination with ara-A for clinical use cannot be selected until information on all of the above factors is available.

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CCNU, Melphalan, Methotrexate, and Prednisone (CAMP) Versus Cyclophosphamide, Methotrexate, and 5-Fluorouracil (CMF) in the Treatment of Advanced Breast Cancer in Postmenopausal Women[1]

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SUMMARY

Sixty-nine postmenopausal patients with advanced breast cancer were randomized to receive treatment with cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) or CCNU, melphalan, methotrexate, and prednisone (CAMP). Response rate (partial and complete remission) was significantly higher with CMF (50%) than with CAMP (20%). Hematologic toxic effects were equally pronounced with the two combinations as were the other side effects with the exception of alopecia, which occurred most frequently with CMF.

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One of the most frequently used multiple drug combinations in the treatment of advanced breast cancer is a combination of cyclophosphamide, methotrexate, and 5-fluorouracil (CMF). The response rate with this regimen is 50%–60%, with a median duration of 9 months (1).

In a previous phase II study that included 35 patients, the combination of CCNU, melphalan (Alkeran), methotrexate, and prednisone (CAMP) given orally resulted in a response rate of 54%, with a median duration of 6 months (2).

The present phase III study was undertaken to compare these two regimens in the treatment of postmenopausal patients with advanced breast cancer.

MATERIALS AND METHODS

Consecutive patients admitted to the participating departments were included in the study. Inclusion criteria were as follows. (*a*) Patients had to be postmenopausal and < 68 years of age. Postmenopausal status was defined by at least 12 months of spontaneous menostasia. Patients who had previous hysterectomy or who continued to have bleeding as a result of cyclical hormonal treatment had to be > 50 years of age. Patients having had oophorectomy were considered postmenopausal 6 months after the oophorectomy. (*b*) Patients had to have advanced measurable breast cancer. (*c*) Performance status had to be ≤ 3 (3). (*d*) The patients had to have given their verbal informed consent. Exclusion criteria were as follows: (*a*) previous treatment with the agents being studied; (*b*) previous or concomitant malignant disease, except from skin cancer and cancer of the cervix uteri, stage 0; (*c*) endocrine additive treatment within the last 4 weeks; and (*d*) malignant disease of the breast other than carcinoma.

Patients were randomly allocated (using a stochastic array of numbers, closed envelope system) to one of two treatment groups:

1. CMF (cyclophosphamide orally, 100 mg/m², Days 1–14; methotrexate iv, 40 mg/m², Days 1 and 8; and 5-fluorouracil iv, 600 mg/m², Days 1 and 8; cycle repeated every 4 weeks).
2. CAMP (CCNU orally, 70 mg/m², Day 1; mel-

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TABLE 1.—CMF vs CAMP in advanced breast cancer: patient data

Patient data	CMF group	CAMP group
No. of patients entered	34	37
No. of evaluable patients	34	35
Median age in yrs (range)	58 (46–68)	59 (37–68)
Median disease-free interval in mos (range)	30 (0–168)	24 (0–96)
Previous endocrine therapy (%)	46	58
Median performance status* (range)	1 (0–3)	1 (0–3)

**From* ref 3.

phalan orally, 4 mg/m², Days 1–5; methotrexate orally, 20 mg/m², Days 21 and 24; and prednisone orally, 40 mg/m², Days 1–5; cycle repeated every 6 weeks).

The treatment was, if possible, continued for at least two cycles. If, at that time, there was progressive disease the patient was removed from the study. Otherwise, the treatment was continued until progressive disease or until relapse after previous remission. If complete remission occurred, treatment was discontinued after 12 months of complete remission.

Pretreatment studies included clinical evaluation, chest roentgenogram, skeletal survey, clinical chemical tests (hemoglobin, wbc and platelet counts, serum calcium, SGOT, and alkaline phosphatase), and estimation of performance status. All visible and palpable lesions were measured to provide a baseline for subsequent examinations. Blood cell counts were repeated on Days 1 and 8 of the CMF cycle and on Days 1 and 21 of the CAMP cycle. The other studies were repeated at intervals of 1–3 months.

If hematologic toxicity occurred, drug doses (except of prednisone) were modified according to the following wbc and platelet counts ($\times 10^3/mm^3$): wbcs > 4 and platelets > 100: 100% dose; wbcs 2.5–4 and/or platelets 75–99: 50% of dose; wbcs < 2.5 and/or platelets < 75: 0 of dose.

Response to treatment was defined according to UICC criteria (3).

RESULTS

Seventy-one patients entered the trial between August 1976 and June 1978. Thirty-four patients were randomized to receive treatment with CMF and 37 patients were randomized to receive treatment with CAMP. Two patients treated with CAMP were lost to followup.

As shown in table 1, the patients receiving the two regimens were comparable with regard to age, performance status, disease-free interval, and previous endocrine therapy.

Overall treatment results are shown in table 2; 50% of the patients achieved objective remission (partial and complete remission) with CMF compared to 20% with CAMP ($P < 0.05$, Mann-Whitney rank sum test, unpaired data). Median duration of response was 9 months for CMF and 6 months for CAMP ($P > 0.10$).

Table 3 also shows that CMF is more active than CAMP irrespective of localization of the disease.

Comparison of survival has not been attempted in the two groups because most of the patients with progressive disease received different secondary regimens.

The hematologic values during treatment are shown in table 4. Leukopenia and thrombocytopenia were equally distributed in the two groups. The other side effects (nausea, vomiting, and stomatitis) occurred at an equal rate in the two groups. However, loss of hair to the extent of requiring a

TABLE 2.—CMF vs CAMP in advanced breast cancer: overall treatment results

Patient response*	No. of patients (%)		*P* value
	CMF group	CAMP group	
PD	9 (26)	15 (43)	
NC	8 (24)	13 (37)	
PR	11 (32)	4 (11)	
CR	6 (18)	3 (9)	
Total	34 (100)	35 (100)	
PR + CR	17 (50)	7 (20)	< 0.05
Median duration of CR + PR in mos (range)	9 (2–13+)	6 (2–10+)	> 0.10

*PD = progressive disease, NC = no change, PR = partial remission, CR = complete remission.

TABLE 3.—CMF vs CAMP in advanced breast cancer: response (partial + complete remission) related to dominant disease site

Dominant disease site	CMF group*	CAMP group*
Soft tissue	6/12	3/7
Bones	2/5	1/9
Viscera	9/17	3/19

*No. of patients responding/No. in group.

TABLE 4.—CMF vs CAMP in advanced breast cancer: % of patients with hematologic toxic effects

Wbc and platelet counts ($\times 10^3/mm^3$)	CMF	CAMP
Wbcs		
> 4.0	11	18
2.5–4.0	34	48
1.5–2.5	31	22
< 1.5	23	12
Platelets		
> 100	73	56
75–100	11	22
50–75	8	10
< 50	8	12

wig occurred in 16 patients treated with CMF as compared to only one patient treated with CAMP.

DISCUSSION

Our results with CMF are in agreement with larger series in the literature (1). In the present study CMF was significantly superior to CAMP both with regard to response rate and duration of response in patients with advanced breast cancer. It should also be emphasized that the superiority of CMF compared with CAMP was observed with equal hematologic and gastrointestinal toxic effects.

The CAMP regimen was chosen because it was easy to administer, and in our phase II trial with CAMP response was obtained in 19 of 35 (54%) of the patients. The disagreement between the results from that previous phase II and the present study remains unexplained.

The CAMP regimen is easy to administer but apart from no alopecia this combination is as toxic as CMF. Thus, considering both efficacy and toxicity, the CAMP regimen is inferior to CMF.

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Bleomycin Alone or Combined With Mitomycin C in Treatment of Advanced or Recurrent Squamous Cell Carcinoma of the Vulva[1]

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SUMMARY

Twenty patients with advanced and/or recurrent vulvar carcinoma were treated with bleomycin alone or combined with mitomycin C. Five of 11 patients in the bleomycin-treated group showed objective response (two complete and three partial) and five of nine patients in the bleomycin-mitomycin C-treated group showed objective response (one complete and four partial). No severe side effects were seen. Now that activity against this disease has been demonstrated for bleomycin, alone or combined with mitomycin C, attention should be directed to treating those patients not cured by surgery and radiation therapy alone.

[Cancer Treat Rep 64:639–642, 1980]

Cancer of the vulva is a rare disease, constituting only 3%–4% of all gynecologic malignancies. It is a disease of old age with an average accession incidence maximum at the age of 65. The treatment of choice is radical vulvectomy with dissection of the inguinal and femoral lymph nodes, as well as external and internal iliac lymph nodes in selected cases. In our clinic this radical surgery is combined with preoperative radiotherapy to the inguinal nodes, and in large lesions to the vulva region. The results of this therapy model are good. The 5-year tumor death rate for all stages is about 35%.[3]

Because of the good primary treatment results, recurrent carcinoma of the vulva is an uncommon disease. Patients with this disease are also likely to be older and thus subject to physiologic impairments which often contraindicate chemotherapy. Therefore, the number of published systemic evaluations of different chemotherapy schedules is small and includes only small numbers of patients.

There are some data on the use of doxorubicin (Adriamycin) (1–3), bleomycin (1,3,5–15), cyclophosphamide (16,17), cytembena (18), 5-fluorouracil (19), 6-mercaptopurine (19), methotrexate (19–22), mitomycin C (19), nitrosoureas (23), and porfiromycin (24); for review, see Deppe et al (25). It is only doxorubicin, bleomycin, and cytembena which allow any conclusion considering the responses: four of six, 28 of 44, and four of 26 patients, respectively.

Thus far there are only eight reports on the use of combination chemotherapy (16,25–30).[4] Turner's combination regimen, bleomycin, vincristine, and methotrexate, proved highly effective in advanced patients; however, it produced toxic effects in some of these patients.[4] Of seven patients in Turner's series, two died within 1 month of treatment, and only one of the remaining five did not respond to treatment. We report here on two regimens, bleomycin alone and bleomycin combined with mitomycin C in primarily advanced or recurrent squamous cell carcinoma of the vulva.

MATERIALS AND METHODS

Two patients with primarily advanced cancer of the vulva and 18 with recurrent and/or distant metastatic cancer of the vulva were selected on the

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[3]Tropé C, and Johnsson JE. Carcinoma of the vulva. An analysis of 237 patients with histologically verified squamous cell carcinoma. Manuscript in preparation.

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[4]Turner R. Paper read at the Bleomycin Meeting, Hammersmith Hospital, London, England, Oct 1975.

basis of the following criteria: evaluable lesions; disease not previously treated by surgery and/or radiotherapy; leukocytes $\geq 3.0 \times 10^9$/liter and platelets $> 100 \times 10^9$/liter; and Karnofsky performance index ≥ 50. All the 20 patients had squamous cell carcinoma. Eighteen of the patients had previously received external-beam radiotherapy and had had a radical vulvectomy. Two patients had such advanced disease that chemotherapy was used as the primary treatment regimen.

The bleomycin regimen used was 15 mg im twice a week up to a total dose of ≤ 300 mg. The combination bleomycin-mitomycin C regimen used was: bleomycin, 5 mg iv during 6–8 hours on Days 1–7; and mitomycin C, 10 mg iv on Day 8 as a bolus dose. This dose schedule was given as one course repeated four times with a 1-week recession period between each course.

Initial examination included physical examination, cbc, ECG, lung roentgenogram, and gynecologic palpation. When indicated, by symptoms or known metastases, skeletal surveys and isotope scans of the liver, brain, and skeleton were performed.

The criteria for response was defined as follows: *complete remission (CR)*—total disappearance of all measurable lesions for at least 1 month; *partial remission (PR)*—at least 50% reduction of measurable tumor; *static disease (SD)*—0–49% decrease of measurable tumor volume and no new lesions; and *progressive disease (PD)*—increasing volume of tumor or new lesions during treatment.

RESULTS

Five of 11 patients in the bleomycin-treated group showed objective response (two CRs and three PRs). The two patients with CR responded for 4 and 24+ months, respectively, and the three patients with PR responded for 2, 2, and 3 months. Median survival for responders was 13 months compared to 3 months for nonresponders. Three of the responders had a recurrence and the other two had distant metastases (table 1).

Five of nine patients in the bleomycin-mitomycin C-treated group showed objective response (one CR and four PRs). Two patients had SD. The patient with CR is still responding after 7 months. Three of the four patients with PRs are still responding after 2+, 3+, and 7+ months. Two of the responders received bleomycin-mitomycin C as the primary treatment. Two of the responders had recurrence of primary tumor and one patient had recurrence in distant metastases (table 1).

Toxicity

In the bleomycin-treated group one patient developed fibrosis of the lung, two developed urticaria, and three developed fever which responded well to symptomatic treatment. In the bleomycin-mitomycin C group three patients developed fever (during the bleomycin treatment), one developed reversible pneumonitis, and four developed moderate thrombocytopenia. No lethal side effects were seen.

DISCUSSION

It is not possible to cure a patient with vulvar carcinoma with chemotherapy administered in a recurrent or metastatic situation. Thus, chemotherapy in situations like this is a palliative treatment, and pronounced side effects can certainly not be accepted, since the patients often are old and subject to physiologic impairments.

Deppe et al (25) reported a promising regimen in four patients treated with doxorubicin. Three of their four patients obtained regression for 28+, 31+, and 32 months without complications. Guthrie (31) and the present authors have also treated patients with vulvar cancer with doxorubicin in conventional doses but with severe toxicity and without any success. Edsmyr (13) reported a $> 50\%$ tumor reduction in two of three patients with advanced vulvar cancer using bleomycin with only moderate side effects.

Encouraged by the Edsmyr's results and the unsatisfactory results obtained previously in our clinic in recurrent vulvar cancer, we decided to try bleomycin as a single drug in treating these patients. Five of 11 of our patients responded and the incidence of toxicity was low. The prolongation of life in the responders was significant compared with the nonresponders.

Miyamoto et al (32) renewed the interest for chemotherapy with combination bleomycin and mitomycin C therapy for advanced squamous cell carcinoma of the cervix. We started their regimen in advanced and recurrent cervical cancer and obtained good results and almost no side effects. Considering these promising results we decided to undertake a phase II trial of advanced and/or recurrent vulvar carcinoma trying to improve our bleomycin results. Though it is too early to state anything definitive about the duration of remission and survival or to compare the data with bleomycin alone or other regimens, the response rate of five of nine patients is promising. The palliative effect of the bleomycin-mitomycin C regimen was good and most

TABLE 1.—Pretreatment features and response to bleomycin and bleomycin-mitomycin C therapy

Patient No.	Age (yrs)	Histology	Clinical stage		Prior treatment*	Duration of remission after 1st treatment (mos)	Recurrence		Total dose (mg) of—		Response	Duration (mos) of—	
			FIGO	TNM			Primary tumor	Distant metastases	Bleomycin	Mitomycin C		Remission	Survival
					Bleomycin alone								
1	68	Well-differentiated	I	$T_1N_0M_0$	Op + RT	216	+	—	300		CR	24+	24+
2	78	Well-differentiated	IV	$T_3N_0M_0$	Op + RT	7	+	—	170		CR	4	13
3	81	Moderate differentiated	IV	$T_3N_2M_0$	Op + RT	7	+	—	285		PR	3	15
4	63	Moderate differentiated	IV	$T_3N_2M_0$	Op + RT	1	+	Pelvis	150		PR	2	12
5	70	Well-differentiated	III	$T_2N_2M_0$	Op + RT	—	+	Inguinal nodes	300		PR	2	3
6	68	Well-differentiated	IV	$T_4N_2M_0$	RT	—	+	Pelvis	300		SD	4	4
7	43	Well-differentiated	I	$T_1N_0M_0$	Op + RT	12	–	Pelvis Skeleton	105		PD	—	11
8	70	Well-differentiated	I	$T_1N_0M_0$	Op + RT	96	+	Pulmonary	180		PD	—	6
9	62	Well-differentiated	IV	$T_4N_1M_0$	Op + RT	8	+	Pelvis Inguinal nodes	180		PD	—	3
10	52	Moderate differentiated	I	$T_1N_0M_0$	Op + RT	3	–	Pulmonary Ribs	45		PD	—	2
11	68	Moderate differentiated	I	$T_1N_0M_0$	Op + RT	11	–	Inguinal nodes	180		PD	—	1
					Bleomycin-mitomycin C combination								
12	70	Well-differentiated	III	$T_1N_2M_0$	Op + RT	6	–	Pelvis	175	50	CR	7+	7+
13	70	Well-differentiated	III	$T_3N_0M_0$	Op + RT	10	+	—	175	50	PR	7+	7+
14	78	Moderate differentiated	III	$T_3N_0M_0$	—	—	–	—	175	50	PR	3+	3+
15	71	Low differentiated	IV	$T_4N_3M_3$	—	—	–	—	140	40	PR	2+	2+
16	70	Well-differentiated	IV	$T_3N_2M_1$	RT	—	+	Inguinal nodes	175	50	PR	3	5
17	77	Well-differentiated	III	$T_2N_2M_0$	Op + RT	6	–	Inguinal nodes	105	30	SD	3	3
18	70	Well-differentiated	II	$T_2N_0M_0$	Op + RT	132	+	—	175	50	SD	8	10
19	70	Moderate differentiated	IV	$T_3N_2M_0$	Op + RT	—	+	Inguinal nodes	70	20	PD	3	3
20	49	Moderate differentiated	I	$T_1N_0M_0$	Op + RT	2	+	—	175	50	PD	—	7+

*Op = vulvectomy + superficial inguinal lymphadenectomy; RT = radiotherapy of the inguinal lymph nodes (^{137}Cs, peak-absorbed dose = 21 Gy).

of the patients led an almost normal life. In contrast to patients with cervical cancer, responses were observed both in sites of local recurrence and in distant metastases; responses in patients with cervical cancer have usually been limited to responses in distant and metastatic sites.

Now that activity against this disease has been demonstrated for different cytostatic drugs, it should be possible by careful consideration to combine the various modalities of treatment, thereby providing a hope of longer survival and, ideally, a higher cure rate.

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Distribution and Elimination of Melphalan in Rats and Monkeys and Distribution in Tumors of Mice Bearing L1210 or P388 Leukemias Sensitive and Resistant to This Agent[1,2]

Robert K. Brown, Glenn Duncan, and Donald L. Hill[3,4,*]

SUMMARY

Following iv injection, melphalan was eliminated monophasically from rat serum (half-life = 0.87 hour) and monkey serum (half-life = 1.9 hours) and in rat bile (half-life = 2.4 hours) and monkey urine (half-life = 1.3 hours). In rat bile and monkey urine, 2% and 20% of the dose, respectively, was excreted in 12 hours as unchanged melphalan. At each time of tissue assay (0.5–4 hours after injection), rat spleen contained less melphalan than serum, liver, or kidneys. The kidneys and bile of monkeys contained more melphalan than serum, liver, or spleen. Only a small amount of radioactivity from labeled melphalan appeared in the feces of monkeys. Melphalan reached higher concentrations in implants of P388 and L1210 leukemia cells sensitive to melphalan than in cell lines resistant to this drug. Furthermore, the amounts of radioactivity bound to macromolecules of the sensitive tumors were higher.

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Melphalan is an important chemotherapeutic agent, effective in the treatment of multiple myeloma (1,2) and as adjuvant chemotherapy for stage II breast cancer (3,4). We have developed a procedure (5) involving high-pressure liquid chromatography (HPLC) for separation of melphalan from its degradation products and have used it in studies on the absorption, distribution, and elimination of this compound in dogs (6).

In the present report, we have extended this work by examining the distribution and elimination of melphalan in rats and monkeys. We have also studied its in vivo distribution in implanted (mouse) leukemia cells sensitive and resistant to melphalan. Preliminary reports of portions of this work have appeared (7,8).

MATERIALS AND METHODS

[^{14}C]melphalan, labeled in the mustard side chain, was provided by the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. It was pure both chemically and radiochemically as determined by HPLC and liquid scintillation counting techniques. Concentrations of dosing solutions were 1 mg/ml in 11 mM HCl and had specific activities of 5.8 microcuries/mg for rats and mice and 8.1 microcuries/mg for monkeys.

Doses of [^{14}C]melphalan were administered iv: 10 mg/kg for mice, 1.6 mg/kg for rats, and 0.75 mg/kg for monkeys. For mice and rats, the doses are 0.8 and 0.6 of the LD10, respectively (F. M. Schabel, unpublished results and ref 12). The dose for the monkey is equivalent to about 0.5 of the expected LD10 on a milligram-per-square-meter basis. The maximum-effective and maximum-tolerated single ip dose for mice bearing L1210 and P388 leukemias

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is 15 mg/kg. An ip dose of 10 mg/kg kills 4.2 logs of sensitive L1210 cells, 1.4 logs of resistant L1210 cells, 5.3 logs of sensitive P388 cells, and 1.7 logs of resistant P388 cells, all of which have been implanted ip (M. W. Trader and F. M. Schabel, Jr, unpublished results). The precise effect of the same dose, administered iv, on cells implanted sc is not known.

Male Sprague-Dawley rats weighing 180–280 g were used in the study of tissue distribution, and rats weighing 410–485 g were used in the study of biliary excretion. The larger rats allowed easier bile duct cannulation and produced greater volumes of bile. Female rhesus monkeys, weighing 4–6 kg, were isolated in metabolism cages for several days before the studies began. All animals were fasted overnight prior to dosing.

Forty male BDF_1 mice received implants of melphalan-sensitive L1210 tumors on the left side and melphalan-resistant tumors on the right side (9). Another 40 male BDF_1 mice received implants of melphalan-sensitive P388 tumors on the left side and melphalan-resistant tumors on the right side. At the time of the experiment, the tumors weighed 400–500 mg. At the appropriate time after dosing, each mouse was anesthetized with diethyl ether and killed. Tumors were removed, weighed, and assayed for melphalan as described below.

For collection of bile, rats were anesthetized with diethyl ether and restrained on a dissection board. The common bile duct was cannulated with polyethylene tubing (inside diameter, 0.86 mm), and the collection receptacle was placed in an ice bath. The femoral vein was exposed to permit injection of melphalan at the time the animal started to regain consciousness.

In tissue distribution studies, rats were anesthetized, restrained on a dissection board, and dosed by way of the femoral vein. At the appropriate times, they were killed by decapitation. Only blood (for serum) was collected from those rats killed 5 and 15 minutes after dosing, whereas blood, liver, kidneys, and spleen were collected from those killed at other times. Two monkeys wre used in tissue distribution studies. Following iv injection of melphalan, they were killed by exsanguination; blood, liver, kidneys, spleen, and bile were collected. Four monkeys were used to determine the serum levels and urinary excretion of melphalan. Prior to these studies, each monkey was fitted with a urinary catheter, and control blood and urine samples were collected.

Total radioactivity in biologic samples was determined using duplicate or triplicate portions processed in a Packard Tri-Carb oxidizer. The $^{14}CO_2$ was trapped in 12 ml of Carbosorb, to which was added 13 ml of Permafluor V (Packard Instrument Co, Downers Grove, IL). Recovery of radioactivity in the scintillation cocktail was essentially 100%, as determined by use of standards.

Protein-free filtrates for HPLC analysis were prepared by adding 2 vol of 10% perchloric acid in 2-methoxyethanol:0.1% acetic acid (55:45, vol/vol) to weighed samples of tissue or fluid and homogenizing with a Brinkmann Polytron apparatus equipped with a P-10 generating head (Brinkmann Instruments, Westbury, NY). Urine samples were prepared for analysis, with a 0.2 vol of perchloric acid solution if the sample was clear and with 2 vol if it contained blood. In addition to precipitating macromolecules from the samples, perchloric acid provided an acid environment in which melphalan was more stable (6). All samples were then centrifuged at 1640 × *g* for 5 minutes. The supernatants were removed, and a 0.5 vol of 1 M KH_2PO_4, was added to precipitate the perchlorate. Samples were filtered under 3 atmospheres of nitrogen through a Millipore filter with a 25,000 molecular weight cutoff (Millipore Corp, Bedford, MA) and stored at –20°C. The extent of binding of the drug to macromolecules of tumor tissue was determined by washing precipitates with 2.5 ml of 2-methoxyethanol:0.1% acetic acid (55:45, vol/vol), then oxidizing to CO_2 and measuring the radioactivity.

The HPLC system employed in analysis of tissue filtrates consisted of two solvent pumps, a solvent-flow programmer, a μBondapak C_{18} column, and a Model 440 ultraviolet detector set at 254 nm (all from Waters Associates, Milford, MA). Ultraviolet profiles were recorded on a stripchart recorder (Omniscribe, Houston Instruments). Pump A delivered a solution of 2-methoxyethanol:0.1% acetic acid (10:90 [vol/vol] for monkey samples and 30:70 [vol/vol] for rat samples) and pump B delivered the same two reagents in a 55:45 (vol/vol) ratio for both species. The solvent program utilized a step gradient which changed from 15% solvent B to 85% solvent B at 3 minutes after injection of the sample. The rate of solvent flow across the column was 1.5 ml/minute. After injection of each sample, 48 consecutive 0.5-ml fractions of the column eluent were collected and analyzed for radioactivity. Melphalan was identified and measured as described previously (6).

Half-lives for melphalan in the various biologic fluids were determined as described previously (6).

RESULTS

Melphalan, injected iv into rats, disappeared from serum monophasically with a half-life of 0.87 hour (fig 1). The apparent volume of distribution

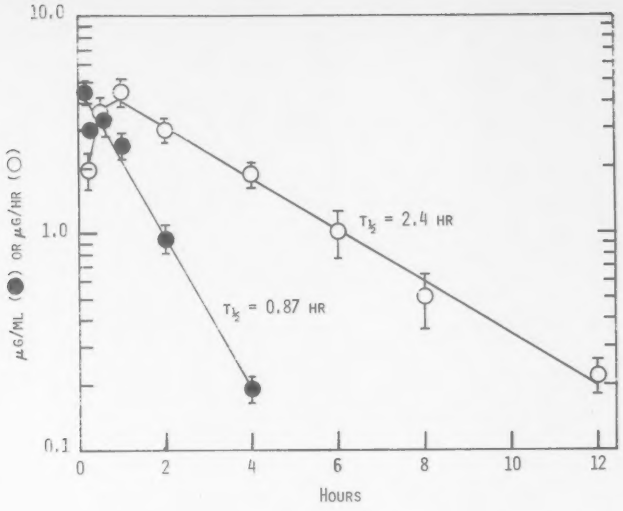

FIGURE 1.—Serum levels and biliary excretion of melphalan in rats. For each time point, data are shown as means ± SE for 4 rats. ○ = bile; ● = serum. Different rats were used in the 2 determinations.

was 0.3 liters/kg, indicating that the drug did not distribute evenly throughout the bodies of these animals. The content of melphalan in serum samples decreased from about 80% of the total radioactivity present at the early times of assay to 19% of the amount present at 4 hours (not shown). Biliary excretion of melphalan by rats reached a maximum at 60 minutes, then declined monophasically with a half-life of 2.4 hours (fig 1). Within 12 hours, only 2% of the total dose was excreted in this manner. At all times of assay, melphalan comprised only 10% of the radioactivity present in the bile (not shown).

Melphalan was eliminated monophasically, from the serum of monkeys, with a half-life of 1.9 hours (fig 2). An earlier phase of about 0.1 hour was not statistically validated. The volume of distribution was 7.5 liters for the monkeys, which weighed about 5 kg each. The percent of total radioactivity representing melphalan in these samples was essentially the same as for rat serum. The rate of urinary excretion of melphalan by monkeys declined monophasically with a half-life of 1.3 hours (fig 2). For each time period, unchanged melphalan represented about 20% of the total radioactivity (not shown).

The distribution of melphalan in selected tissues of rats and monkeys is shown in table 1. At no point was the content of drug in the spleen of rats greater than that in the serum, liver, or kidney. There were no notable differences in the disappearance of the drug in these latter three samples. In monkeys, the levels of melphalan in liver, spleen, and serum were essentially the same. The concentration was, however, considerably higher in kidney and bile, especially in bile at 4 hours.

The percentages of total radioactivity present as melphalan in rat liver and spleen and in monkey serum, kidney, and spleen were similar to those in serum (not shown). In rat kidney and monkey liver and bile, the relative amount of melphalan was less. In rat kidney, the amount dropped from 33% at 0.5 hour to 4% at 4 hours. In monkey liver, 20% of the radioactivity present was melphalan at 2 hours and 9% at 4 hours; and in monkey bile 9% was melphalan at 2 hours and 8% at 4 hours (not shown).

An average of 8% of the total radioactivity administered was eliminated in the feces of monkeys within 13 days (not shown). The assay was not appropriate for determination of unchanged melphalan in feces.

The distribution of melphalan in implanted tumors in mice was examined. Melphalan reached higher concentrations in the sensitive P388 leukemia cells of mice than in the resistant line. Concentrations were also higher in sensitive L1210 leukemia cells at 0.25 and 2 hours (table 2). The

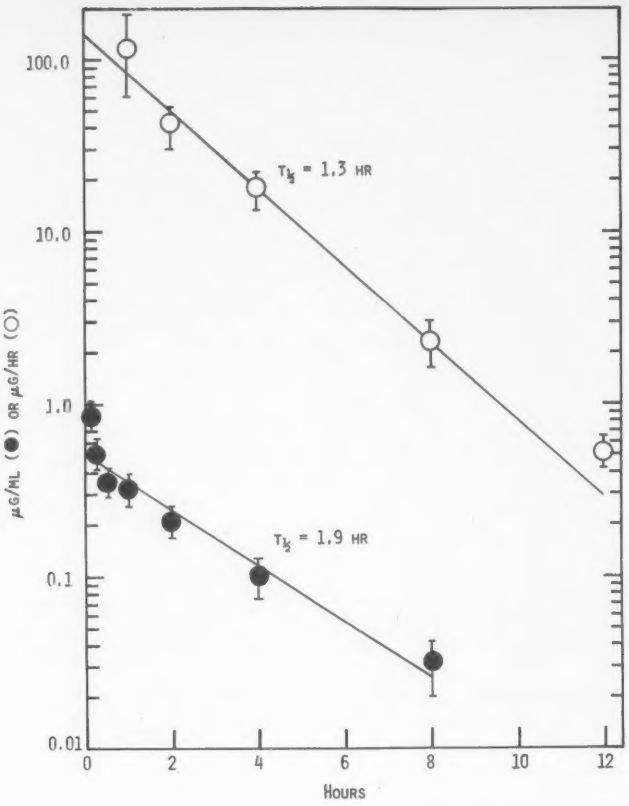

FIGURE 2.—Serum levels and urinary excretion of melphalan in monkeys. For each time point, data are shown as means ± SE for 4 monkeys. ○ = urine; ● = serum. The same monkeys were used for the 2 determinations.

TABLE 1.—Distribution of melphalan in serum and selected tissues of rats and monkeys

Tissue	Distribution of melphalan (µg/g or ml) in —					
	Rat*				Monkey†	
	0.5 hr	1 hr	2 hrs	4 hrs	2 hrs	4 hrs
Serum	3.26 ± 0.26	2.58 ± 0.13	0.94 ± 0.08	0.19 ± 0.03	0.22	0.10
Liver	3.13 ± 0.26	2.44 ± 0.26	1.24 ± 0.08	0.27 ± 0.07	0.21	0.08
Kidney	3.58 ± 0.26	2.59 ± 0.26	1.51 ± 0.10	0.32 ± 0.07	2.57	0.83
Spleen	1.17 ± 0.04	0.89 ± 0.03	0.55 ± 0.05	0.13 ± 0.02	0.20	0.08
Bile	ND‡	ND	ND	ND	2.63	3.89

*Values are mean ± SE for 4 rats at each time period.
†Values are for individual monkeys.
‡ND = not determined.

TABLE 2.—Melphalan concentrations in sensitive and resistant tumors implanted in the same host

	Melphalan concentrations (μg/g of tumor)* in—			
	L1210		P388	
Hrs	Resistant	Sensitive	Resistant	Sensitive
0.25	1.03 ± 0.19	3.33 ± 0.61†	1.00 ± 0.06	1.43 ± 0.14†
0.5	1.57 ± 0.16	1.50 ± 0.11	0.70 ± 0.04	1.28 ± 0.08†
1	0.68 ± 0.14	1.07 ± 0.31	0.36 ± 0.04	0.73 ± 0.06†
2	0.15 ± 0.02	0.33 ± 0.09†	0.11 ± 0.01	0.25 ± 0.03†

*Values are mean ± SE for 5 individual mice.
†$P < 0.05$ that the value for the sensitive tumor is higher than that for the resistant.

TABLE 3.—Radioactivity from [^{14}C]melphalan bound to macromolecules of sensitive and resistant tumors implanted in the same host

	Radioactivity from [^{14}C]melphalan (μg/g dry wt)* in—			
	L1210		P388	
Hrs	Resistant	Sensitive	Resistant	Sensitive
0.25	4.08 ± 0.27	4.06 ± 0.36	3.28 ± 0.09	4.75 ± 0.19†
0.5	4.95 ± 0.47	6.57 ± 0.52†	4.85 ± 0.29	7.62 ± 0.45†
1	7.79 ± 0.73	10.94 ± 0.87†	5.62 ± 0.29	11.08 ± 0.36†
2	7.44 ± 0.68	12.93 ± 0.70†	7.17 ± 0.41	14.68 ± 0.36†

*Values are mean ± SE for 5 individual mice.
†$P < 0.05$ that the value for the sensitive tumor differs from that for the resistant.

percentage of total radioactivity present as melphalan was essentially the same in extracts of sensitive and resistant tumors, decreasing from 20% at 0.25 hour to 10% at 2 hours (not shown). The amount of radioactivity bound to macromolecules of sensitive tumors was higher (except for the L1210 tumor at 0.25 hour) than that found in the resistant tumors (table 3).

DISCUSSION

Elimination of melphalan from serum of rats (half-life = 0.87 hour) and monkeys (half-life = 1.9 hours) is a monophasic process, at least to the limit of sensitivity of the present assay. Mice similarly demonstrate a single elimination phase with a half-life of 0.68 hour (5). Dogs, however, show a biphasic elimination with half-lives of 0.24 and 1.1 hours (6); and man demonstrates a biphasic pattern with a second half-life of 2.0 hours (10) or 1.1 hours (11). Although serum half-lives of drugs are generally longer in larger species, it is unlikely, due to the chemical instability of melphalan, that its second half-life in any species will be > 2 hours.

Biliary excretion does not appear to provide a major means for elimination of melphalan in rats since it accounts for only a small portion of the dose. This process may be more important in dogs, which have much higher concentrations of melphalan in their bile at times up to 4 hours after an iv injection (6). Although fecal excretion of radioactivity derived from [^{14}C]melphalan was demonstrated in monkeys, urinary excretion appears to be the primary means by which unchanged melphalan is eliminated from the body.

Disappearance of melphalan from kidney and liver roughly paralleled its elimination from serum. The high concentrations of melphalan in these tissues and the low apparent volume of distribution imply that other tissues would have lower concentrations. A relatively low concentration was observed for spleen, although this is a site of toxicity in dogs (12). In monkeys the apparent volume of distribution was high, perhaps due to the fact that kidney tissue concentrations of the drug were tenfold higher than the other tissues examined. In the dog (6) and mouse (5), kidney tissue also contained relatively large amounts of melphalan. In dogs, the melphalan concentration in the spleen was lower than that in the serum (6). This was also demonstrated here in rats.

The major radioactive products present in these

samples were generally the derivatives, formed by hydrolysis, for which one or both of the chlorine atoms of melphalan are replaced by hydroxyl groups (5). The remaining radioactive materials have not been identified.

Active transport of melphalan (13,14) may be faster in cells of some tissues than in others, leading to greater intracellular concentrations of the drug. Such active transport processed may be less effective in the tumor cells resistant to melphalan than in sensitive ones, and may contribute to the resistance of the tumor to chemotherapy by this agent. Consistent with this is the observation here that resistant leukemia cells contained lower levels of melphalan than sensitive cell lines.

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Advanced Diffuse Histiocytic Lymphoma Treated With Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone (CHOP) Without Maintenance Therapy[1,2]

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SUMMARY

Thirty-one patients with stage III or IV diffuse histiocytic lymphoma were treated with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) at 3- or 4-week intervals for at least six cycles or until two cycles after complete remission (CR) was achieved. At the completion of therapy, CR was documented by examination of sites known to previously contain disease. After documentation of CR, patients were followed without maintenance therapy. Fifteen patients (48%) achieved CR, four (13%) had equivocal lymphangiograms on restaging but were otherwise free of disease, two (6%) died of unrelated causes during therapy, two (6%) developed central nervous system lymphoma during therapy and despite peripheral remission eventually died of the CNS disease, and eight (26%) had either partial or no response. Twelve of the 15 patients achieving CR remain disease-free with no therapy for 9+ to 43+ months. Three patients relapsed at 3, 4, and 23 months after discontinuation of therapy. Three of the four patients with equivocal restaging lymphangiograms remain without progressive disease with no therapy for 10+ to 24+ months. The projected median survival time for all patients is 41 months. The risks of long-term (maintenance) chemotherapy are considerable and include the induction of acute nonlymphoblastic leukemia. CHOP without maintenance therapy is able to induce long-term disease-free survival (and probable cure) in a significant number of patients with advanced diffuse histiocytic lymphoma.

[Cancer Treat Rep 64:649–654, 1980]

The previously poor prognosis for tumors designated diffuse histiocytic lymphoma (DHL) has improved dramatically following the use of combination chemotherapy (1). It is now apparent that patients with advanced DHL who achieve complete remission (CR) with combination chemotherapy frequently do not relapse during extended followup despite the absence of maintenance therapy (2). One of the regimens effective in inducing CR in advanced DHL is the combination of cyclophosphamide, doxorubicin (Adriamycin), vincristine, and prednisone (CHOP), reported by McKelvey et al (3). However, the administration of 18 months of maintenance therapy makes it difficult to compare the efficacy of CHOP in inducing long-term CRs with that of other regimens reported in the literature. Table 1 shows five previous series of patients with DHL treated with combination chemotherapy that resulted in 77 CRs not followed by maintenance

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TABLE 1.—Duration of remission in diffuse histiocytic lymphoma

Authors (ref No.)	No. of patients achieving CR	No. of relapses	No. of relapses after 1 yr of unmaintained remission
Anderson et al (2)	29	6	0
Cadman et al (4)	12	6	1
Durant et al (5)	28	13	2
Weinerman et al (6)	8	5	2
Totals	77	30 (39%)	5 (16%)

therapy. Approximately one third of the patients relapsed, with 84% of the relapses coming within 1 year of discontinuing therapy. To see if remissions induced by CHOP are equally durable, we treated 31 patients having stage III and IV DHL with the CHOP regimen and administered no maintenance therapy to patients achieving CR.

PATIENTS AND METHODS

Thirty-one patients with stage III or IV DHL treated between July 1975 and July 1978 form the basis of this report. The diagnosis was confirmed by review of the slides using the criteria of Rappaport (7). Stages were assigned in accordance with the Ann Arbor staging system (8).

The treatment regimen included cyclophosphamide at a dose of 750 mg/m² iv on Day 1, doxorubicin at a dose of 50 mg/m² iv on Day 1, vincristine at a dose of 1.4 mg/m² (maximum dose, 2 mg) iv on Day 1, and prednisone at a dose of 100 mg orally daily on Days 1–5. This program was repeated at 21- or 28-day intervals. A minimum of six cycles of therapy and at least two cycles past disappearance of clinically evident disease were to be administered. At the completion of therapy, sites known to previously contain disease were re-evaluated and, whenever possible, re-biopsied.

CR was defined as the absence of demonstrable disease after the completion of therapy and restaging. Partial remission (PR) was defined as a > 50% regression of all measurable disease lasting at least 1 month, but lack of documentation of a CR. One subgroup of patients who had only an equivocal lymphangiogram on restaging keeping them from CR will be considered separately. The duration of CR was defined as the time from restaging to relapse and the duration of survival was defined as that from the first day of CHOP to death. All patients were evaluable. Survival of groups was calculated using the life-table method. Deaths from tumor or possible treatment-related complications were treated similarly. Statistical significance was determined using log-rank analysis when comparing life tables and the Fischer's exact test when comparing response rates of groups.

RESULTS

The clinical characteristics and results for these 31 patients are listed in table 2. Fifteen patients (48%) achieved CR; four (13%) had equivocal lymphangiograms on restaging but were otherwise free of disease; two (6%) died of unrelated causes (ie, one with congestive heart failure (CHF) and one with an aorto-duodenal fistula with no tumor at autopsy) during therapy in clinical remission; two (6%) developed CNS lymphoma during therapy and despite peripheral remission eventually died of the CNS disease (autopsy of one of the patients showed no peripheral tumor); four (13%) had transient PRs but their disease progressed and they died of lymphoma despite further therapy; and four (13%) did not respond to CHOP and rapidly died. The life table for survival of the entire group is shown in figure 1. Fifty-seven percent of the patients are predicted to be alive at 2 years and the predicted median survival time is 41 months.

Three of the patients achieving CR have relapsed: one at 3 months, one at 4 months, and one at 23 months. The other patients remain in CR from 9+ to 43+ months. One of the patients (No. 17) who had a CR except for an abnormal restaging lymphangiogram received maintenance therapy with cyclophosphamide, vincristine, and prednisone for 8 months and the other patients have been followed without additional therapy. Three of the patients with equivocal lymphangiograms on restaging currently remain in remission with no treatment 10+ to 24+ months. One patient (No. 19) developed progressive disease at the site of the lymphangiogram abnormality 6 months after discontinuing therapy. It is possible that some of the three patients who had equivocal lymphangiograms but are still in remission are in fact disease-free but have false-positive lymphangiograms. The superiority of survival for patients achieving CR or missing CR only because of an equivocal lymphangiogram over

TABLE 2.—Patients with DHL treated with CHOP

Patient No.	Age/ sex	Previous treatment	Stage of DHL	Stage IV site	No. of cycles of CHOP	Treatment result	Remission duration (mos)	Survival (mos)
				Complete responders				
1	64/M	—	IVA	Lung	7	CR	43+	50+
2	85/M	—	IVB	Bone, marrow	9	CR	35+	44+
3	68/M	—	IIIA	—	6	CR	37+	43+
4	60/M	—	IIIB	—	6	CR	38+	44+
5	44/M	Radiation therapy	IVB	GI tract	8	CR	35+	43+
6	53/F	—	IIIB	—	9	CR	23	41
7	55/F	Radiation therapy + chemotherapy	IVA	GI tract	5	CR	35+	40+
8	45/F	—	IVA	Pancreas	6	CR	28+	33+
9	57/F	—	IIIB	—	9	CR	3	15
10	47/F	—	IVA	Lung	6	CR	13+	19+
11	68/M	—	IVA	Marrow	6	CR	12+	17+
12	83/M	—	IVA	Testes	6	CR	9+	15+
13	63/F	Radiation therapy	IVA	Bone	7	CR	18+	25+
14	75/F	—	IIIA	—	6	CR	16+	21+
15	65/M	—	IVB	Liver	6	CR	4	14+
				Equivocal lymphangiogram on restaging (otherwise CR)				
16	59/F	—	IIIA	—	11	?	24+	34+
17	68/M	—	IVB	Bone	11	?	21+	31+
18	61/F	—	IIIA	—	6	?	10+	20+
19	56/F	—	IIIB	—	9	?	6	17+
				Died during therapy without evidence of lymphoma				
20	59/F	Radiation therapy	IVA	Cervix and nasopharynx	5	—	—	5+
21	65/M	—	IVB	GI tract	4	—	—	4+
				CNS lymphoma developing during therapy (peripheral remission)				
22	59/M	Radiation therapy	IVA	Bone	5	—	—	23
23	54/M	—	IVB	Bone	9	—	—	17
				Partial responders and nonresponders				
24	65/M	—	IVB	Skin	8	PR	—	9
25	58/M	—	IVB	Bone	5	PR	—	8
26	33/M	—	IVB	Bone	6	PR	—	6
27	78/F	—	IVB	Pancreas	6	PR	—	10
28	66/M	Radiation therapy	IVB	Pleura	2	NR	—	2
29	57/M	—	IVB	Marrow, liver	1	NR	—	1
30	50/F	—	IVB	Marrow	2	NR	—	2
31	53/F	—	IVB	GI tract	1	NR	—	1

patients with lesser responses is highly significant (fig 1).

A number of factors have been identified in previous reports to have prognostic significance in patients with advanced DHL. These include stage IV versus stage III (9), symptom status B (10), gastrointestinal (GI) tract involvement (11), bone marrow involvement (9), a large primary tumor (12), and CNS involvement (13). Our patients without systemic symptoms (nine CRs among 13 patients) had a significantly superior survival (fig 2) compared to those with symptom status B (six CRs

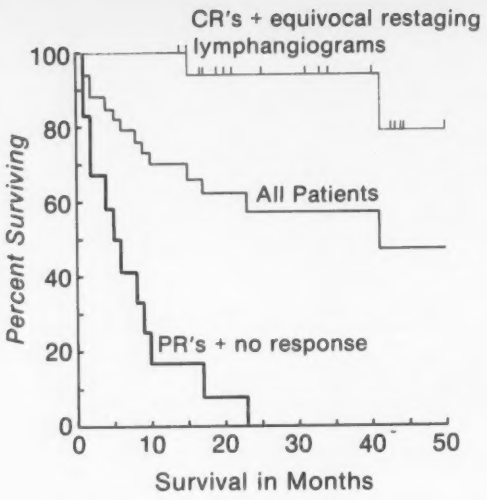

FIGURE 1.—Survival of patients with CR or those lacking CR only because of an equivocal restaging lymphangiogram vs those with PR or no response. Vertical lines = patients still alive. Upper and lower curves are significantly different at $P < 0.001$.

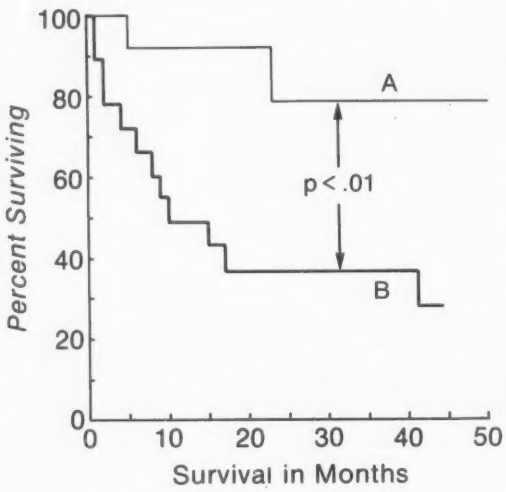

FIGURE 2.—Survival of patients who had symptom status A (eg, without fever, night sweats, or weight loss) vs those who had symptom status B (eg, with fever, night sweats, or weight loss).

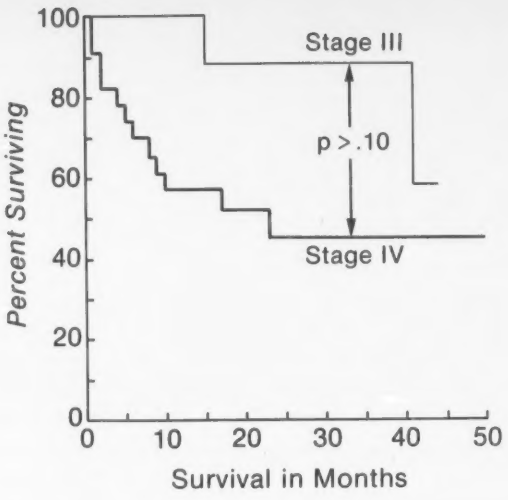

FIGURE 3.—Survival of patients who had stage III disease vs those who had stage IV.

among 18 patients ($P < 0.01$). The survival of patients who had stage III DHL (five CRs among eight patients) was marginally but not significantly better (fig 3) than those who had stage IV (ten CRs among 23 patients). Patients with previous therapy (three CRs among six patients) did as well as those treated initially with CHOP (12 CRs among 25 patients). Patients with GI involvement (two CRs among four patients) and bone marrow involvement (two CRs among four patients) did not have significantly poorer outcomes. No patient had CNS lymphoma at the initiation of therapy. A report describing the significance of a large primary tumor appeared after this study had been in progress for several months, and retrospective review of operative reports did not always reveal the diameter of the tumors to have been accurately recorded.

The toxicity of this regimen was significant. Eight of the 31 patients required dose reduction because of granulocytopenia during therapy with cyclophosphamide and doxorubicin. There were two episodes of sepsis, requiring hospitalization and parenteral antibiotics, from which the patients recovered. There were no deaths from infection or hemorrhage. Four patients required a reduction in dosage of vincristine because of paresthesia or mild motor weakness. There were no episodes of severe, disabling neurotoxic effects. One patient required

discontinuation of cyclophosphamide because of hemorrhagic cystitis. Three patients developed CHF during therapy. In each case co-existent coronary artery disease was proven or suspected and two patients had received previous thoracic radiation. The relative contribution of each of the factors to the CHF was difficult to determine in retrospect. Two of the three patients died and in both cases CHF was felt to be a factor contributing to their death.

DISCUSSION

The CR rate was 68% utilizing CHOP in DHL in a study reported by McKelvey et al (3). Although this is higher than we observed, intensive restaging evaluations were not required. A longer followup of that study is now available and McKelvey has reported a 61-week median remission duration (for responding patients) and a 44-week median survival (for all patients) for patients with histiocytic lymphoma treated with CHOP followed by maintenance therapy (14). These results are not superior to our findings using CHOP without maintenance therapy.

As it has become increasingly apparent that certain tumors can be permanently eradicated by drug therapy alone, the value of less aggressive maintenance therapy following vigorous remission induction therapy has been questioned. When a careful "restaging" evaluation to rule out residual disease is carried out at the completion of a planned course of therapy, relapse is unusual in DHL (2). Since other treatment regimens using many of the same agents have been able to produce cures in DHL without maintenance therapy, we felt that the CHOP regimen would likely be equally effective. Results of this study suggest that this has, in fact, been the case.

The advantages of avoidance of extended therapy with cytotoxic agents are considerable. Each cycle carries with it the dangers of acute myelosuppression, and with subsequent cycles, cumulative myelosuppression is a danger. In addition to the acute problems of infection and bleeding, it has become increasingly apparent that extended therapy with alkylating agents incurs a small but real risk of the development of acute nonlymphoblastic leukemia (15). If extended therapy offers no increased chance of cure, then it is best avoided.

Despite these encouraging results, it is important to note that one half or more of treated patients still die. Fourteen of the 31 patients in this series are dead, 12 as a direct result of progressive lymphoma. Failure of drugs to cure a cancer can be because of primary drug resistance (failure to induce remission), secondary drug resistance (relapse developing after initial response), relapse in a sanctuary site, or death due to treatment toxicity. In our series, eight patients failed to achieve a CR initially and all of these are dead. Only three patients relapsed after documented CR. Two patients failed to achieve remission solely because of the development of CNS lymphoma. Both patients had bone involvement, which has been previously suggested to predict for CNS relapse (13). Two patients died with CHF during initial therapy, and death might have been related to doxorubicin toxicity. Thus, it appears that the major reason for treatment failure is primary drug resistance. DHL is heterogenous morphologically (16) and immunologically (17), and these results suggest biologic heterogenity. It may be that certain as yet unidentified subtypes will require different drugs or different therapeutic modalities to effect sustained remissions.

It is now apparent that advanced DHL is a potentially curable neoplasm when treated only with cytotoxic chemotherapy. It has been previously demonstrated that regimens designated COPP (1), COMA (4), BCOP (5), and BACOP (18) are capable of inducing durable remission when treatment is administered intensively for only a few cycles past disappearance of clinically obvious disease. Our results suggest that the CHOP regimen is also capable of inducing long-term, disease-free survival without maintenance therapy.

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Phase II Study in Advanced Sarcoma: Randomized Trial of Pyrazofurin Versus Combination Cyclophosphamide, Doxorubicin, and *cis*-Dichlorodiammineplatinum(II) (CAP)[1,2]

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SUMMARY

Twenty-eight patients with various histologic types of measurable metastatic sarcoma were randomly assigned to receive treatment with combination cyclophosphamide, doxorubicin (Adriamycin), and *cis*-dichlorodiammineplatinum(II) (CAP) or the single-agent, pyrazofurin. Patients who failed one arm of the protocol were eligible for cross-over treatment. Six of 24 patients treated with CAP achieved objective tumor regressions (25%), with a median duration of 11 months. None of 21 patients receiving pyrazofurin achieved a response. CAP is an effective, well-tolerated combination for the treatment of advanced sarcoma.

[Cancer Treat Rep 64:655–658, 1980]

Metastatic or advanced soft tissue sarcomas not amenable to surgical or radiotherapeutic control have not generally been responsive to chemotherapy. Although polychemotherapy employing doxorubicin (Adriamycin), vincristine, cyclophosphamide, and DTIC or actinomycin D has been reported to produce substantial regressions of moderate duration in this group of malignancies (1,2), our experience with these agents has generally been disappointing (3,4). Therefore, we felt that further treatment trials with combination chemotherapy and single new agents were warranted.

Single-agent *cis*-dichlorodiammineplatinum(II) (CDDP) has a wide spectrum of antitumor activity, including that against advanced sarcoma (5). Furthermore, it is synergistic with doxorubicin (6) and cyclophosphamide (7), agents active against sarcomas, and because of a unique toxicity spectrum, it can be administered in combination with these agents without dose modification. Antitumor activity has been observed with pyrazofurin (PRZF) in animal models of sarcoma (8), and in early human studies tumor regression was noted in one patient with sarcoma.[4] This report summarizes our experience with the combination of cyclophosphamide, doxorubicin, and CDDP (CAP) and the single-agent, PRZF, in patients with advanced sarcomas.

MATERIALS AND METHODS

Twenty-eight patients with advanced inoperable sarcomas were randomized to one of the two protocol treatment programs described below. Each patient had an expected survival of > 8 weeks, a wbc count of > 4000/ml, a platelet count of > 100,000/ml, and no elevation of direct bilirubin. Patients with a serum creatinine level of ≥ 1.3 mg/dl or with a history of cardiac disease were initially excluded from the CAP combination. Patients previously treated with any of these agents were not eligible for the CAP arm of the protocol. All patients had measurable disease and were stratified by performance status and presence of cardiac or renal disease.

Seventeen men and 11 women with a mean age of 46.6 years were treated. Twenty-four patients (86%) had overt visceral metastatic disease (22 had

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pulmonary disease and two had hepatic disease). Only two patients had received previous chemotherapy and a third patient had been treated with transfer factor. Histologic subtypes of primary tumors included synovial cell sarcoma, liposarcoma, and osteogenic sarcoma (four cases each), malignant fibrous histiocytoma and leiomyosarcoma (five cases each), rhabdomyosarcoma and undifferentiated mesenchymal sarcomas (two cases each), and fibrosarcoma and spindle cell sarcoma (one case each).

The CAP combination was administered as follows: doxorubicin, 15 mg/m² iv; cyclophosphamide, 150 mg/m² iv; and CDDP, 15 mg/m² by rapid iv infusion without hydration or mannitol on Days 1–3. The single-agent, PRZF, was administered at a dose of 240 mg/m² iv on Day 1. Both regimens were repeated at 28-day intervals with appropriate dose modification until the disease progressed or until toxicity or cumulative dose limits (500 mg/m² for doxorubicin) precluded further treatment with the programmed therapy.

Prior to each cycle of treatment the patients were re-evaluated with a physical examination, chest roentgenogram, ECG, and hematology and chemistry blood groups. Interval wbc and platelet counts were obtained twice weekly. If the wbc or platelet count nadirs were < 1500 or 50,000/ml, respectively, drug doses were reduced by 33% at the subsequent administration. If no significant leukopenia or thrombocytopenia occurred, ie, no wbc or platelet count fall to < 3000 or 100,000/ml, respectively, the dose was increased by 25% in the absence of other significant toxicity. CDDP was decreased by 50% if the creatinine rose to 1.3–1.6 mg/dl and by 75% if the creatinine rose to 1.7–1.9 mg/dl. CDDP was discontinued if the creatinine was ≥ 2.0 mg/dl, but was reinstituted at a 50% dose when levels returned to 1.5 mg/dl. PRZF was reduced by 33% when moderate dermatitis or stomatitis occurred.

Patients who experienced tumor progression while receiving PRZF were candidates for secondary treatment with the CAP combination if the study requirements were met. Patients who failed the combination chemotherapy could be treated with PRZF if they were still treatment candidates.

A partial regression was defined as a 50% reduction in the product of the longest perpendicular diameters of the indicator lesions with no progression of other lesions. A complete regression was defined as the disappearance of all evidence of sarcoma. Duration of regression was calculated from the start of chemotherapy until evidence of disease progression. Progression was defined as a 25% increase in the product of the longest perpendicular diameters of the indicator lesions over pretreatment measurements or the appearance of new disease.

RESULTS

Three of 13 (23%) patients who were initially treated with the CAP combination achieved objective tumor regression, but none of 15 patients initially treated with PRZF alone achieved remission. The mean duration of regression was 13 months. Seventeen patients with progressive disease were crossed-over to secondary treatment regimens. Three of 11 (27%) patients in this group achieved an objective regression with CAP (mean duration of regression, 18+ months) (table 1). No tumor regression was observed in six patients receiving secondary treatment with PRZF. No further patients were treated with PRZF after 21 consecutive progressions with no evidence of tumor regression. Five additional patients in the total group of 24 patients receiving CAP (21%) had stability of disease for at least 5 months (mean, 7 months), while in all patients receiving PRZF, disease progressed within 90 days of initiation of treatment and in 19 of 21 disease progressed within 60 days. Median survival was 8.3 months for patients treated initially with CAP while the median survival time was 6 months for patients treated with PRZF; this is not a significant difference.

Common toxic effects from the CAP regimen which occurred in most patients were nausea, vomiting, and alopecia. One patient refused further treatment because of gastrointestinal toxicity, despite 9 months of disease stability. Transient elevation of serum creatinine occurred in one of 24 patients receiving this regimen. No clinically detectable hearing impairment was noted and no instance of cardiotoxicity was observed. Six patients (25%) developed mild to moderate stomatitis. Leukopenia (≤ 3000 cells/ml) developed in 18 of the 24 patients (75%) and thrombocytopenia (≤ 100,000 cells/ml) developed in six (25%). Doses were escalated in two of the six patients who did not have myelosuppression. Three of these six patients had marked early disease progression (two with wbc count nadirs < 3500/mm³), and further treatment was not felt to be justified. The sixth patient has significant stomatitis with scheduled doses. All patients achieving regression had leukopenia.

Toxicity was much less in the PRZF-treated patients. Mild to moderate nausea was noted in 35% of the patients, and vomiting was noted in 15% (three of 21 patients). Mild alopecia was observed in two patients. Five patients had moderate stomatitis and two had peri-anal pruritus and mild

TABLE 1.—Response and duration by diagnosis in patients treated with CAP

Cell type	Total No. of patients	Objective tumor regression		Disease stability > 3 mos	
		No. of patients	Duration of response in mos (response type)	No. of patients	Duration of response in mos
Synovial cell sarcoma	4	2	8 (partial regression), 11 (partial regression)	0	5,9
Liposarcoma	4	2	19 (partial regression),* 26+ (complete regression)	2	6,10‡
Malignant fibrous histiocytoma	5	1	10 (partial regression)	0	
Osteogenic sarcoma	4	1	20 (complete regression)†	0	
Leiomyosarcoma	2	0		2	
Rhabdomyosarcoma	1	0		0	
Spindle cell sarcoma	1	0		0	
Fibrosarcoma	1	0		1	6
Undifferentiated mesenchymal sarcoma	2	0		0	
Totals	24	6		5	

*Doxorubicin was discontinued after 11 mos; cyclophosphamide and CDDP were continued until disease progression.
†Chemotherapy was discontinued after 12 mos upon doxorubicin tolerance in complete regression.
‡Patient refused further therapy after 9 mos; disease progression within 60 days.

dermatitis. Two patients (10%) had leukopenic nadirs as defined above.

DISCUSSION

A 25% objective regression rate (six of 24 patients) was achieved with CAP therapy when it was used as primary or secondary treatment. The mean duration of regression was 16+ months, with one complete regression continuing at 26+ months. All patients who achieved regression and the five patients who had disease stability of ≥ 5 months had leukopenic nadirs. No severe toxicity was noted and generally the program was well-tolerated.

Previous trials using polychemotherapy for advanced soft tissue sarcomas have reported regression rates as high as 55%–60% when employing cyclophosphamide, vincristine, doxorubicin, and DTIC every 3 weeks (1,2) or doxorubicin and DTIC alone every 3 weeks (9). These optimistic figures must be interpreted with caution considering the relatively high percentage of patients who were disqualified (12%–32%) in each study and the lack of randomized control characteristics. Evaluation of the same or similar programs by different investigators has frequently failed to substantiate these results. When alternating cyclophosphamide and vincristine with doxorubicin and DTIC or when combining all four drugs every 4 weeks, Pinedo et al (10) obtained only a 23%–37% regression rate. Baker et al (11) achieved objective tumor regression in 22%, 25%, and 35% of their patients employing doxorubicin and DTIC; doxorubicin, DTIC, and antinomycin D; and cyclophosphamide, doxorubicin, and DTIC, respectively, every 3 weeks. Our results with three programs consisting of DTIC, doxorubicin, and vincristine every 5 weeks; actinomycin D, cyclophosphamide, and vincristine every 5 weeks or an intensive-therapy program consisting of DTIC, doxorubicin, actinomycin D, and cyclophosphamide have produced regression rates of only 11%, 8%, and 21%, respectively, with significant resulting toxicity (3,4).

Direct comparison of results of these studies is not possible, however, because of the heterogeneity of this group of tumors and the various doses and treatment intervals employed. Indeed, higher drug doses used more frequently may increase regression rates but have been associated with increased gastrointestinal and hematologic toxicity.

The results of this study indicate that CAP is an effective antitumor regimen for advanced sarcoma and is comparable to treatment programs previously reported. Furthermore, the duration of regression (mean, 16+ months; median, 11 months) compares favorably to our previous experience with polychemotherapeutic endeavors (median duration, 3–6 months) (3,4) and those of others (Foley et al [12], 5 months; Beretta et al [9], 9 months). Patient tolerance of the CAP regimen reported herein was quite acceptable.

No regressions were noted among 21 consecutive

patients receiving PRZF as a primary or secondary treatment; thus, we conclude that further investigations using PRZF in patients with advanced sarcoma do not appear warranted.

The lack of significant difference in median survival time between those patients initially treated with CAP and those treated with PRZF (8.3 and 6 months, respectively) is most likely a reflection of rapid disease progression in patients receiving PRZF and early cross-over (ie, 19 of 21 patients within 60 days) to the more effective regimen.

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Relative Pulmonary Toxicity and Antitumor Effects of Two New Bleomycin Analogs, Pepleomycin and Tallysomycin A[1]

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With the assistance of **Susan T. Arnold, Adrienne Dishman, Jacqueline R. Honig, and Tamara D. Schanwald**

SUMMARY

The relative therapeutic and toxic effects of two new analogs were compared with bleomycin over a range of doses. Therapeutic effects were determined in mice bearing Lewis lung carcinoma and B16 melanoma. On a milligram-per-kilogram basis, tallysomycin A was two to three times as potent as bleomycin in inhibiting the growth of these two experimental solid tumors in vivo. However, tallysomycin A was also two to three times as potent as bleomycin in producing pulmonary toxic effects. Lethality and skin toxicity were similarly increased. Pepleomycin, on the other hand, was approximately equivalent to bleomycin in antitumor potency but exhibited significantly less pulmonary toxicity. Despite this decreased lung toxicity, pepleomycin was more lethal, with 50% and 100% mortality at doses which produced 0 and 19% mortality, respectively, in mice treated with bleomycin. Pepleomycin also had a peculiar central nervous system toxicity characterized by hyperirritability and persistent gyrating movements of the animals. Thus, there are distinct quantitative as well as qualitative differences in the therapeutic and toxic properties of these two analogs compared to bleomycin.

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Bleomycin, a mixture of glycopeptides, is clinically useful for the treatment of lymphomas, testicular carcinomas, and squamous cell carcinomas (1–3). The drug is particularly valuable in combination-chemotherapy regimens because of its notable lack of bone marrow toxicity. However, the total dose which can be administered is limited by potentially fatal pulmonary toxicity (4,5). We have developed a model of bleomycin lung toxicity in mice and have shown that it is reproducible and dose-dependent, that it can be quantified by measurement of lung collagen content as estimated by hydroxyproline levels, and that continuous infusion of the drug decreases pulmonary toxicity compared to that when equivalent doses are administered by bolus injection (6,7). In the present study, we have used this model, as well as the transplantable murine solid tumors, Lewis lung carcinoma and B16 melanoma, to study the therapeutic and toxic properties of two new analogs of bleomycin, pepleomycin, and tallysomycin A.

Pepleomycin is a semisynthetic analog isolated from a fermentation broth of *Streptomyces verticillus* enriched with phenylethylaminopropylamine, which constitutes the terminal amine portion of pepleomycin (fig 1) (8). Preliminary studies of this compound indicated that it retained the antibiotic and antitumor properties of bleomycin in screening tests while apparently producing less pulmonary

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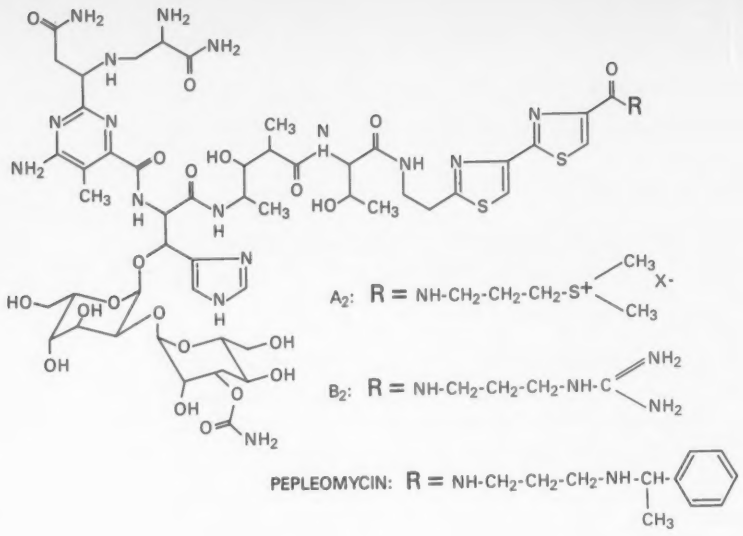

FIGURE 1.—Structural formulas of bleomycins A_2 and B_2 and pepleomycin.

fibrosis in mice as indicated by histologic examination (9,10). This compound is currently undergoing phase I and II clinical trials in Japan.

Tallysomycin A, a glycopeptide isolated from an unusual strain of actinomycetes, has had broad antibiotic and antitumor activities in preliminary screening tests. It is structurally similar to bleomycin (11,12), differing from bleomycin by the presence of a hydroxyl on the carbon adjacent to the bithiazole group in the bleomycinic acid core of the molecule, to which is also attached a talose sugar (fig 2). This compound is of particular interest because it involves a major structural modification from bleomycin, has a greater affinity for DNA in vitro, and is more potent in preliminary screening tests in microbial and animal tumor systems (13,14).

MATERIALS AND METHODS

Animals

Male BDF_1 mice (Hazelton Laboratories, Inc, McLean, VA), weighing 20–25 g at the start of the experiments, were used throughout the study. They were maintained on Purina Rodent Chow and water ad libitum. Animals not bearing tumors were used in experiments to assess the pulmonary toxicity of bleomycin. For studies of antitumor activity of the drugs, BDF_1 mice were implanted sc with either Lewis lung carcinoma or B16 melanoma as described below.

Studies on Therapeutic Effects

The antitumor properties of all three drugs against both Lewis lung carcinoma and B16 melanoma were compared in male BDF_1 mice. The tumors were minced, and a suspension containing 10^7 tumor cells per milliliter was prepared. Cell viability was determined by trypan blue dye exclusion, and 10^6 cells, or 0.1 ml, were injected sc into the left flank of each animal. Treatment was begun on Day 4 after implantation, with daily sc injections of drug for 10 days. The effects of treatment were measured by determining the length and width of the primary tumor at 5-day intervals, and by recording the time of survival from implantation of tumor.

Drugs

Bleomycin, as the mixture of glycopeptides (Lot U11A2), and pepleomycin were obtained from Nippon Kayaku, Inc, Japan, through the courtesy of Dr. John Douros, Natural Products Branch, National Cancer Institute, Bethesda, MD. Tallyso-

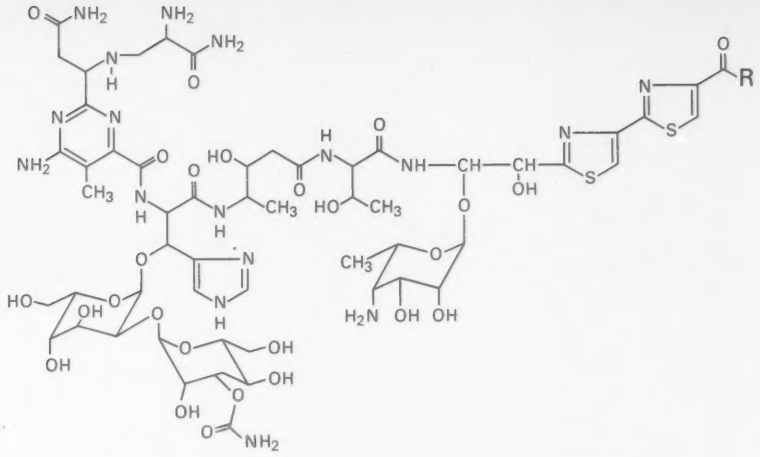

FIGURE 2.—Structural formula of tallysomycin A.

mycin A was obtained as a gift from Bristol Laboratories, Inc. The drugs were dissolved in 0.9% saline and administered sc in the right flank. Control animals received 0.9% saline sc.

For studies of pulmonary toxicity, drugs were administered twice weekly sc for 4 weeks, and animals were killed 8 weeks after the initiation of treatments. The bleomycin doses were 5, 10, and 15 mg/kg, with the highest dose known from previous studies to produce significant increases in lung hydroxyproline content and 10%–20% mortality from pulmonary toxicity (6,7).

These same doses were used for pepleomycin. However, the highest dose of pepleomycin, 15 mg/kg, produced 100% mortality over 8 weeks of observation. Therefore, two additional dose groups of 6.5 and 8.0 mg/kg were added to pepleomycin treatment groups of 5, 10, and 15 mg/kg, in order to more accurately assess the pneumotoxic dose-response curve for the drug. Eight animals were studied at each dose except at the lethal doses, when 16 animals were treated in each group. Because previous studies had indicated that tallysomycin A was more potent than bleomycin (15), the highest dose of tallysomycin A used in these experiments was 10 mg/kg. Additional groups of animals were treated with tallysomycin A at doses of 1.25, 2.50, 5.0, 6.5, and 8.0 mg/kg.

In studies of therapeutic effects against Lewis lung carcinoma and B16 melanoma, the drugs were administered sc daily for 10 days, beginning on Day 4 after tumor implantation. The following dose groups were included: bleomycin and pepleomycin at 5, 10, and 15 mg/kg; tallysomycin A at 1.25, 2.5, 5.0, and 10.0 mg/kg.

Lung Toxicity

We have previously demonstrated that dose dependent and reproducible increases in lung collagen content occur in chronic bleomycin pulmonary toxicity in mice, as measured by whole-lung hydroxyproline levels (6). In experiments assessing lung toxicity, non-tumor-bearing mice were treated twice weekly with a range of doses of each drug and were killed 8 weeks after the initiation of treatment. Lung collagen content was estimated by measuring the hydroxyproline content of whole lungs of each mouse according to the method of Woessner as previously described (6,16).

RESULTS

Therapeutic Effects

All three drugs demonstrated significantly prolonged survival of mice implanted with B16 melanoma (fig 3). In this experiment, every treatment

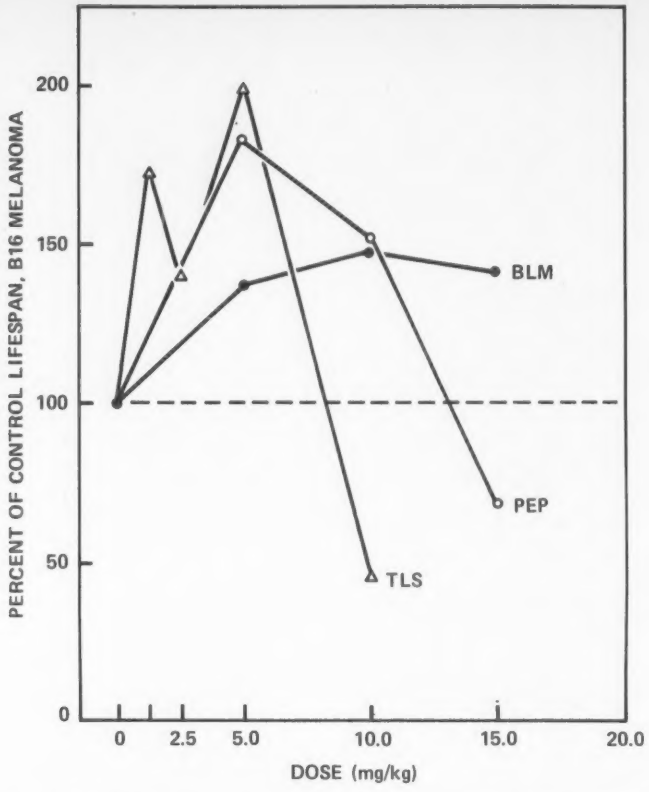

FIGURE 3.—Effects of bleomycin (BLM), pepleomycin (PEP), and tallysomycin A (TLS) at various doses on lifespan of mice bearing B16 melanoma. Effects of all treatments were significantly different from controls ($P < 0.05$, 8 mice/dose).

group was significantly different from controls, with the highest dose of both tallysomycin A and pepleomycin exhibiting lethal drug toxicity and resulting in significantly decreased lifespans. However, at the intermediate drug dose of 5 mg/kg daily for 10 days, both analogs were significantly superior to bleomycin in prolonging lifespan. In fact, the lowest tallysomycin A dose, 1.25 mg/kg, produced an increase in lifespan of 73%, which was superior to that produced by any of the three dose levels of bleomycin. However, differences in inhibition of growth of the primary tumor were not as apparent, (fig 4). It is evident that pepleomycin inhibits B16 melanoma growth similarly to bleomycin whereas tallysomycin A is approximately three times as potent in inhibiting the growth of this tumor on a milligram-per-kilogram basis.

None of these three drugs produced significant increases in lifespan of mice bearing Lewis lung carcinoma in the doses and schedules employed (data not shown). However, marked inhibition of primary tumor growth was observed (fig 5). Again, tallysomycin A was two to three times as potent as bleomycin in inhibiting the growth of Lewis lung carcinoma, but pepleomycin was slightly less potent. At the highest dose of pepleomycin, 15 mg/kg, all animals died before Day 20 after implantation, which is attributed to lethal toxicity since controls were still alive at that time. For each of the three drugs inhibition of tumor growth continued for approximately 1 week after the cessation of treatment, but from Day 20 to Day 25 the rate of primary tumor growth was similar to that in control animals.

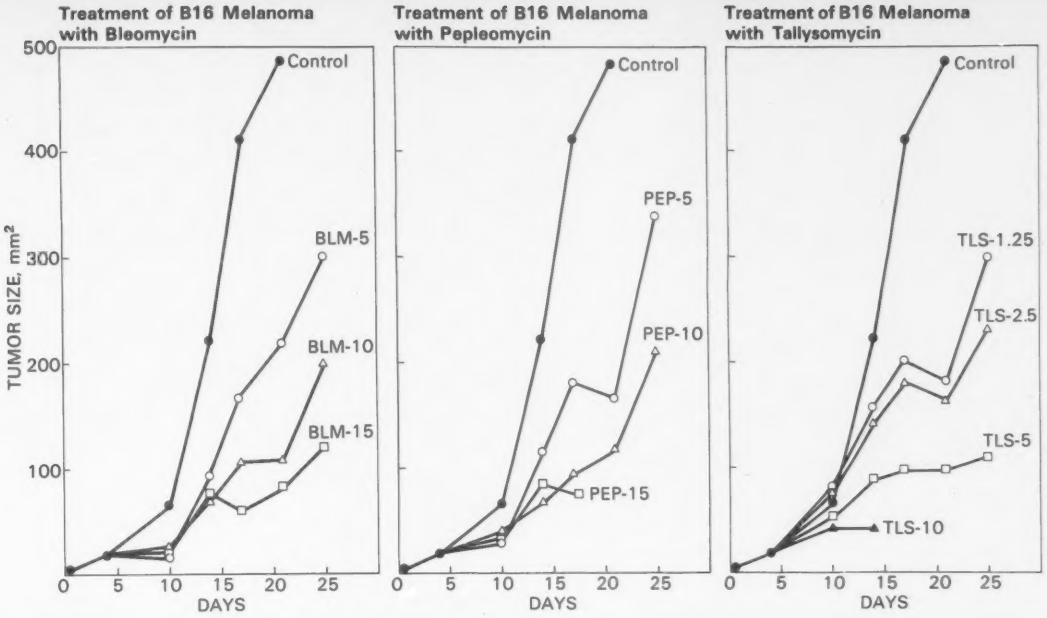

FIGURE 4.—Tumor size (expressed as the product of length × width) in mice bearing B16 melanoma and treated with bleomycin (BLM), pepleomycin (PEP), or tallysomycin A (TLS). The doses indicated (mg/kg) were administered ic daily for 10 days beginning 4 days after tumor implantation.

Toxicity

Measurements of lung hydroxyproline levels after treatment with a range of doses show considerable differences in lung toxicity among pepleomycin, tallysomycin A, and bleomycin (fig 6). At one intermediate dose of pepleomycin, 6.5 mg/kg twice weekly for 4 weeks, a significant increase in lung collagen content was observed. However, none of the other doses of pepleomycin demonstrated increases in lung hydroxyproline content compared to control animals treated with saline, although the highest dose of pepleomycin, 15 mg/kg, produced 100% mortality before the 8-week time point at which lung toxicity was measured. Bleomycin, as expected, produced significant and dose-dependent increases in hydroxyproline levels at each of the three doses employed. Tallysomycin A, on the other hand, was more potent than bleomycin in producing pulmonary toxic effects at both 5 and 10 mg/kg. At the 5-mg/kg dose, ten of 16 animals treated with tallysomycin A developed whole-lung hydroxyproline levels of > 250 μg, whereas only five of 16 animals treated with bleomycin at the same dose developed increases in lung hydroxyproline > 250 μg.

Comparison of lethality during the 8 weeks of treatment and observation is presented in the lower part of figure 6. The highest dose of tallysomycin A used, 10 mg/kg, produced 50% mortality whereas the same dose of bleomycin produced no mortality and 15 mg/kg of bleomycin produced 19% mortality. Gross autopsy of these animals showed consolidation of both lungs consistent with prior histologic observations that at these doses and schedules pulmonary toxicity is the cause of death for animals treated with bleomycin. The lungs of animals dying after high doses of pepleomycin grossly appeared slightly hyperemic, but they did not show the consolidative lesions observed with both tallysomycin A and bleomycin. On the basis of lethality and lung hydroxyproline content over a range of doses, we conclude that tallysomycin A is approximately two to three times as potent as bleomycin in lung toxicity. Tallysomycin A produced pulmonary fibrosis at a dose level of 5 mg/kg which was equivalent to between 10 and 15 mg/kg of bleomycin (fig 6) (6). Furthermore, the LD50 of tallysomycin A given twice weekly for 4 weeks was 10 mg/kg, while for bleomycin it was 25–30 mg/kg (6). For both drugs the cause of death at these doses and schedules is pulmonary toxicity.

Both analogs produced skin changes qualitatively

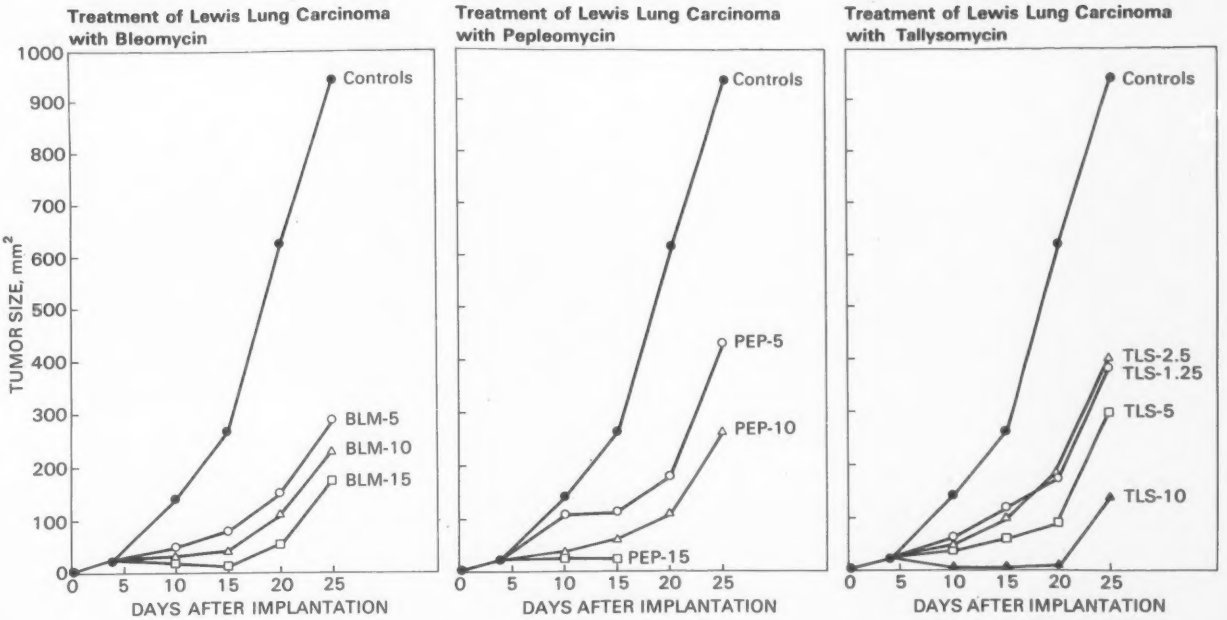

FIGURE 5.—Tumor size (expressed as the product of length × width) in mice bearing Lewis lung carcinoma and treated with bleomycin (BLM), pepleomycin (PEP), and tallysomycin A (TLS). The doses indicated (mg/kg) were administered sc daily for 10 days beginning 4 days after tumor implantation.

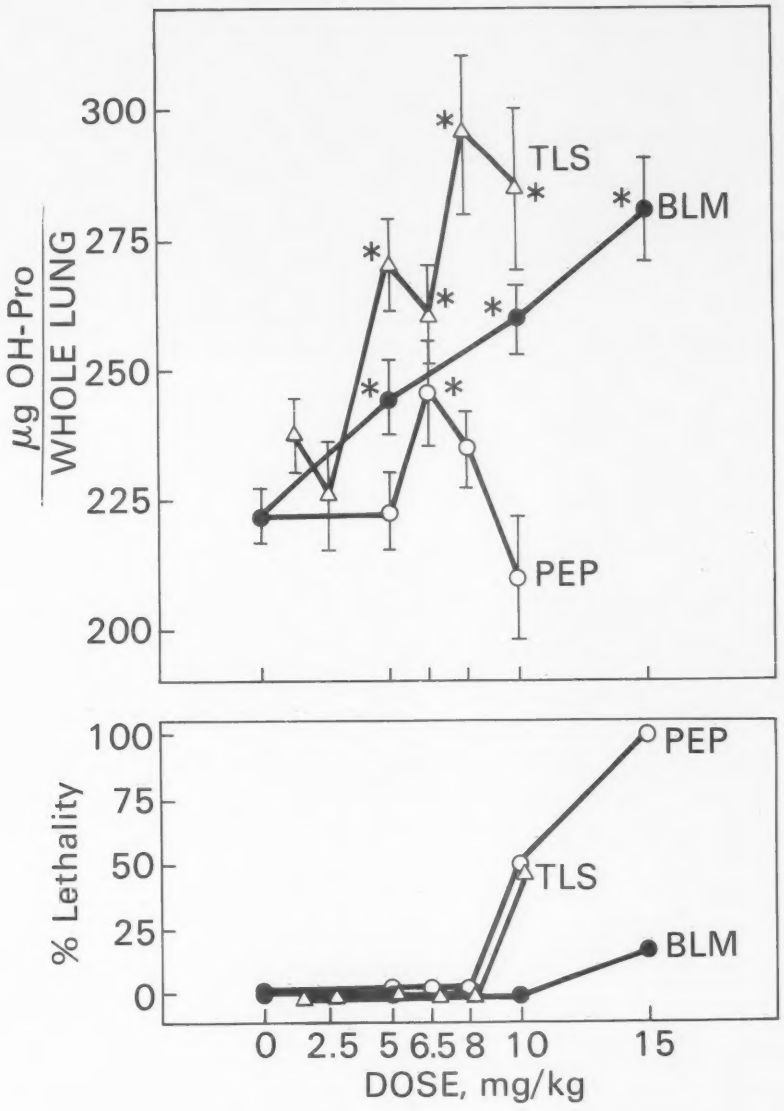

FIGURE 6.—Toxic effects of pepleomycin (PEP) and tallysomycin (TLS) compared to bleomycin (BLM) toxicity. The upper portion describes whole-lung hydroxyproline levels at various doses of the drugs, which were administered sc, twice weekly for 4 wks. The lower portion indicates % mortality over 8 wks of observation after initiation of treatments. * = significantly different from control lung hydroxyproline levels ($P < 0.05$, 8–16 mice).

similar to those produced by bleomycin. Mice treated with pepleomycin developed a peculiar neurologic toxicity, characterized by hyperirritability and gyrating or burrowing rotatory movements when picked up by the tail. Detailed neurologic testing and histopathologic examination of the brain were not performed.

DISCUSSION

Both pepleomycin and tallysomycin A present interesting opportunities to study the structure-activity relationships of the bleomycins. In the case of pepleomycin, the substitution of phenylethylaminopropylamine as a semisynthetic terminal amine adduct produces marked changes in the toxicologic profile of the drug, while it retains similar antitumor potency against Lewis lung carcinoma and B16 melanoma. Pulmonary toxicity is definitely decreased; however, as compared to bleomycin, pepleomycin shows increased lethality and the peculiar CNS toxicity. This drug has produced increased hepatic and renal toxic effects in both mice and dogs as compared to bleomycin in other studies (10), and these toxic effects may contribute to an increase in lethality. These findings re-emphasize the principle that a series of analogs are not necessarily toxic or even lethal by identical toxicologic or pathologic mechanisms.

When an antimicrobial assay was used to compare the organ distribution of pepleomycin in mice to that of bleomycin, it was of great interest that appreciable levels of pepleomycin could be detected in the brain, whereas bleomycin was not detected in the brain (9,10). Neurologic toxicity of pepleomycin has not been observed thus far in phase I and II testing in patients in Japan (M. Ogawa, personal communication). These clinical studies in Japan have also shown a somewhat decreased incidence of pulmonary toxicity with pepleomycin in patients, although skin toxicity is increased and antitumor effects do not appear to be different from those of bleomycin.

Our studies of tallysomycin A indicate that this analog is two to three times as potent as bleomycin both in its therapeutic effects against the two tumors studied and in pulmonary toxicity. This is slightly less than the potency described for tallysomycin A in a previous study in which the acute toxicity was four times that of bleomycin while the antitumor effects were estimated to be four to 12 times greater (15). Preliminary results of a comparison of the chronic pulmonary toxicity of tallysomycin A and bleomycin by other investigators using our mouse model but a slightly different range of doses indicate that tallysomycin A is approximately four times as potent as bleomycin in producing increases in lung hydroxyproline content (17). These discrepancies with our data are minor and illustrate the difficulties in assigning an *exact* ratio for relative potencies of drugs. Such a ratio will vary depending on doses, animal strains, and other factors even when comparing single parameters.

Decreased metabolism of bleomycin by the lungs, compared to other organs, has been proposed to account for the lungs being the major sites of bleomycin toxicity (18,19). In this regard, it would be of interest to determine the relative activities of pepleomycin and tallysomycin A as substrates for the bleomycin-inactivating enzyme, bleomycin hydrolase, in various tissues. We have recently developed a high-performance liquid chromatography assay for measurement of bleomycins and their metabolites (manuscript in preparation), and are currently investigating the role of lung metabolism in the pulmonary toxicity of bleomycin analogs.

The results of this and other studies suggest several areas of interest for possible initial clinical trials in patients. Although pepleomycin may be less pneumotoxic, particular attention should be directed to possible neurologic, hepatic, or renal toxicity. The finding in mice that pepleomycin is distributed to brain tissue indicates that it might be detectable in spinal fluid in man. Although tallysomycin A did not demonstrate an improved therapeutic index over bleomycin in this study, it did show considerable antitumor activity at low doses and thus might be useful in a low-dose continuous infusion treatment schedule.

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Staging of Testicular Cancer: A Proposed Clinical-Surgical Schema[1]

William DeWys,* Franco M. Muggia, and Edwin M. Jacobs[2]

Many different systems have been developed for staging of testicular cancer (1–10). These systems have often been developed by one therapeutic discipline to meet a particular requirement of the treatment strategy of that discipline. As shown in table 1, there is basic agreement in all classifications as to division into three groups. However, there are important differences as to the boundaries of these three divisions. The division between stage I and II is usually between disease limited to the scrotum versus disease spread to the regional nodes. However, in Caldwell's classification (5) invasion through the testicular capsule places a patient in stage II while in other classifications these cases remain in stage I. The division between stage II and III is usually between retroperitoneal disease versus disease spread beyond the retroperitoneal space. However, in Golbey's classification (2) nodes above the diaphragm are included in stage B but labeled B_2, and in Sandeman's classification (4) they are labeled stage II,N_3. In contrast, some investigators (11) include unresected retroperitoneal nodes in stage III.

There are also important differences in the incorporation of information from surgical staging. Boden and Gibb (1) and Golbey (2) do not discuss reclassification for patients whose staging is changed by the results of retroperitoneal node dissection. In two classifications, patients retain their original classification regardless of the results of node dissection (3,4). In these systems, stage I includes patients whose nodes were histologically positive and stage II includes patients with histologically negative nodes. In Maier's system (6), patients whose nodes were clinically negative but biopsy positive were classified IB. Since this system attempts to combine clinical staging (Roman numeral) and surgical staging (alphabet letter), we have designated this a hybrid in table 1. However, this system does not address reclassification of clinical stage II if the nodes are negative at surgery.

As noted above, these staging systems have often been developed to meet the requirements of a particular treatment strategy. With the current emphasis on multidisciplinary treatment (12) a staging system which would be used by all therapeutic disciplines is a desirable goal. We propose a system based on discussions with physicians from multiple disciplines which we believe will be acceptable to all disciplines.

Anatomic Staging

Stage labeling is an exercise aimed at expressing the *anatomic* extent of disease for purposes of planning treatment and estimating prognoses. Accurate labeling of stage is also essential for the conduct of scientifically sound clinical trials. One of the complexities of staging is that the clinically apparent extent of disease may not agree with the surgically defined extent of disease, and these cases may be labeled differently in different staging systems. For example, the patient whose clinically assessed disease was limited to the testes but who was found to have involved nodes at retroperitoneal dissection might be labeled stage IB (6) or IIA (5) or simply stage I or II, depending on the staging system used.

We propose the use of a separate label for clinical staging and for surgical staging (fig 1). These separate labels could be used both to designate the best estimate of the stage of disease at different time points of patient evaluation and also to designate the final stage label at the conclusion of an evaluation. Use of stage labels sequentially during evaluation will be illustrated below under the discussion of serum markers. Use of these staging labels to designate the stage at the conclusion of an evaluation could involve use of only the label giving the more advanced stage or could involve use of two numbers, a clinical stage (C) number and a surgical stage (S) number. Thus, a designation of CI, SII would indicate a patient whose clinical studies were negative but whose surgical staging showed retroperitoneal disease.

Regarding clinical staging one must address the use of the equivocal diagnosis on reports of clinical

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Clinical Staging

CI Limited to testes. Bipedal lymphangiogram, CT scan, and abdominal ultrasound negative

CII Testes plus retroperitoneal nodes involved. Bipedal lymphangiogram, CT scan, or abdominal ultrasound positive

CIII Metastases beyond retroperitoneal nodes

Surgical Staging

SI Limited to testes. All retroperitoneal nodes negative on extensive microscopic study

SII Testes plus retroperitoneal nodes involved. This includes microscopic, gross, and unresectable disease extent

SIII Metastases beyond retroperitoneal nodes

FIGURE 1.—Proposed composite clinical and surgical staging of testicular cancer.

studies. We suggest that our colleagues in diagnostic specialties be requested to qualify the "equivocal" diagnosis to "equivocal, more likely negative" and "equivocal, more likely positive." In this way, each report can be put into either the negative or positive category for analysis.

With regard to clinical stages I and II, one must keep in mind the limitations of the clinical diagnostic tools employed. Lymphangiograms may be expected to have a false-positive rate of 17% and a false-negative rate of 26% in a composite series (13). Specialized centers may have a somewhat lower rate of false positives and false negatives. The group at Memorial Sloan-Kettering (14) reports a false-positive rate of 5% and a false-negative rate of 12%. The lower limit of sensitivity is about 1 cm for CT scan and about 2 cm for ultrasound. These limits of accuracy are clearly recognized in the use of the combination of a clinical and surgical staging system.

Serum Markers

Use of serum markers as an adjunct to staging has recently been discussed (15). Use of markers is based on the observation that 90% of patients with testicular cancer with clinically evident disease will have an elevation of β-human chorionic gonadotropin (HCG), α-fetoprotein (AFP), or both (16). For use of markers as an adjunct to staging, however, it will be important to know the incidence of positive markers for patients with microscopic disease and this likely will be lower than has been observed for clinically evident disease. A cooperative study of stage SI and SII disease, which is currently in progress, should provide this information (13).

Interpretation of serum markers requires consideration of their biologic half-lives (AFP, 5 days and HCG, 18–24 hours). In a patient who has undergone resection of tumor, serial determinations are required. These can be plotted on semilog graph paper to determine the half-life visually, or the equation

$$T_{1/2} = \frac{-0.693 \cdot T}{\log_e\left(\frac{\text{conc } T}{\text{conc } 0}\right)}$$

can be used to calculate the half-life. If the half-life observed in a patient exceeds the known biologic half-life, residual disease must be presumed to be present.

The marker results from peripheral blood, however, do not provide information as to the anatomic localization of residual tumor. A possible remedy to anatomic localization of tumor if other localization techniques fail is use of assay of the alpha subunit of HCG (16).

Based on the above discussion of possible false-negative results, biologic half-life, and the lack of anatomic specificity of serum markers, we conclude that serum markers are best used as an adjunct to staging rather than attempting to integrate marker results into a staging schema. They are most useful in indicating those patients whose stage is underestimated by the composite of other information. Thus, a clinical stage I patient who has a persistently elevated marker must be considered to have stage CII or III while a patient with stage SII who has a persistently elevated marker after retroperitoneal node dissection either has had an incomplete resection of retroperitoneal metastases or has stage III disease.

A notation which could be used to designate this

application of markers as an adjunct to staging for the two examples just cited would be:

$$\text{CI} \rightarrow \text{AFP pos} \rightarrow \text{CII or III}$$
and
$$\text{SII} \rightarrow \beta\text{HCG pos} \rightarrow \text{CIII}.$$

Disease Extent Within Each Surgical or Clinical Stage

In addition to the broad designation of anatomic stage, the extent of disease within an anatomic compartment may have biologic, prognostic, and therapeutic implications. These may be designated by use of the rubric of the TNM system. Several schemata have been proposed (*see* table 1). Our recommendations as to the T and N categories are based on discussions of an interdisciplinary committee of urologists, radiation therapists, and medical oncologists who recently formulated a protocol for adjuvant chemotherapy for stage SII testicular cancer (13).

In the T category (fig 2), the majority of patients will have tumor limited to the testis. This will be designated T_1 and requires no special consideration as to treatment other than inguinal orchiectomy. Invasion of the tunica or epididymis has been correlated with a higher risk of recurrence but this risk of recurrence can be largely related to a higher incidence of nodal metastases (17).[3] If there has been extension of tumor along the spermatic cord to the level of transection of the cord at the inguinal ring (T_2), tumor contamination of the orchiectomy wound may occur and adjuvant radiation or chemotherapy may be considered. If normal scrotal anatomic barriers have been broken (T_3 = tumor invasion of scrotal wall or normal barriers broken by surgery such as prior orchiopexy or scrotal orchiectomy) tumor can spread via the lymphatics of the scrotal wall to the inguinal nodes, and this must be considered in treatment planning.

In the nodal compartment, extent of involvement within a node (microscopic, gross enlargement, or extracapsular extension), the size of the involved nodes, and the number of involved nodes all have prognostic importance (*see* table 1). A schema incorporating these three variables is shown in figure 3. Patients in whom clinical evaluation leads to the conclusion that massive retroperitoneal involvement is inoperable can be included with the N_4 group. Of note, patients with unresectable or partially resectable disease are included in stage II in this schema rather than in stage III as recommended by some investigators (11). However, treatment of unresectable stage II patients should include combination chemotherapy comparable to that used for stage III disease.

A partial documentation of the validity of this proposed classification of the N compartment is shown in table 2.[3] Microscopic involvement (N_1) is associated with increased risk of recurrent disease compared with microscopically negative (N_0) nodes. Patients with gross metastases (N_2 or N_3 of fig 3 schema) have a strikingly higher risk of recurrence than N_1 patients. Since a large number of patients (69% of all node-positive patients) are in this category, subdivision by number of nodes, size of nodes, and extranodal extension (*see* fig 3) should be feasible and worthwhile. Finally, patients with palpable metastases have the worst prognosis, supporting the value of use of size of nodes and resectability status in the schema shown in figure 3.

This proposed schema (fig 3), while most applicable to surgical staging, may show correlations with clinical staging. Specifically, if a metastases is ≥ 2 cm in diameter (stage N_{2b} or higher), one could expect a positive result on lymphogram, CT scan, and/or ultrasound nearly 100% of the time. Also, abdominal disease which is easily palpable on clinical examination will often be found surgically to be in the N_4 category. In contrast, the greatest discrepancy between clinical and surgical staging would be expected for the N_1 category where few cases would be diagnosed as stage II by clinical study.

The UICC-AJC staging of the extent of disease within the metastatic disease (M) category is shown in figure 4. The implications of this schema for surgical management of metastases are apparent with patients in category M_{1b} especially being considered for surgical removal of metastases. The size of the metastases within an organ system is also an important prognostic factor. Samuels et al (11) have used a classification in which metastases < 2 cm in diameter are designated limited while metastases > 2 cm are designated advanced. This classification has been used by others (18–20).

An apparent limitation of the M classification (fig 4) and the Samuels' classification is that neither provides a full accounting of the number of metastases (except for 1 vs 2 or more in the M classification) or of the size of metastases (except for $<$ or $>$ 2 cm in the Samuels' classification). Thus, a patient with a single metastasis of 2.1 cm in diameter would be scored in the Samuels' system as having more advanced disease than a patient with multiple

[3]Batata MA, Chu FCH, Hilaris BS, et al. TNM staging of testis cancer. Presented at the 61st Annual Meeting of the American Radium Society, Los Angeles, CA, Mar 1979.

TABLE 1.—Representative previously proposed staging classifications

Author (ref No.) and discipline*	Clinically limited to testis		Clinically positive nodes		Distant metastases
	Nodes surgically negative	Nodes surgically positive	Nodes surgically negative	Nodes surgically positive	
Boden and Gibb (1) RT,C	A	?†	?†	B	C
Golbey (2) MO,C	A	?†	?†	B_1—nodes below diaphragm	B_2—nodes above diaphragm C—hematogeneous metastases
Hussey et al (3) RT,C	I	I‡	II‡	IIA—moderate size nodes IIB—massive nodes	IIIA—nodes above diaphragm IIIB—extranodal
Sandeman and Matthews (4) RT,C	IT_1—confined to testis IT_2—invading vessel or scrotal wall	I‡	II‡	IIN_1—radiologically positive IIN_2—palpable	IIN_3—nodes above diaphragm $IIIM_1$—intrathoracic $IIIM_2$—beyond lung and mediastinum
Caldwell (5) RT,H	I	IIA	?†	IIA—moderate size nodes IIB—nodes bulky >2 cm	IIIA—mediastinal and supraclavicular IIIB—beyond nodes
Maier and VanBuskirk (6) RT,H	IA	IB	?†	II	III
Staubitz et al (7,8) U,S	I	II	I	II	III
Skinner (9) U,S	A	B_1—<6 nodes encapsulated B_2—> 6 nodes or through capsule	A	B_1—<6 nodes encapsulated B_2—>6 nodes or through capsule	C
Javadpour and Bergman (10) U,S	IA—confined to testis IB—testicular adnexia IC—scrotal wall	IIN_1—microscopic IIN_2—gross IIN_3—capsular invasion IIN_4—unresectable	IA—confined to testis IB—testicular adnexia IC—scrotal wall	IIN_1—microscopic IIN_2—gross IIN_3—capsular invasion IIN_4—unresectable	$IIIM_1$—solitary $IIIM_2$—multiple
Present proposal	CI,SI	CI,SII	CII,SI	CII,SII	III

*Discipline includes: RT = radiation therapy; MO = medical oncology; U = urology and type of staging: C = clinical; H = hybrid, with clinical and surgical elements; S = surgical.

†Original reference does not discuss reclassification based on surgical results.

‡Patients remain in clinical stage regardless of results of node dissection.

T_1 Limited to testes, rete testes, and epididymis

T_2 Invasion of spermatic cord at inguinal ring

T_3 Invasion of scrotal wall (or normal scrotal barriers broken as by orchiopexy or scrotal orchiectomy)

FIGURE 2.—Staging of the primary tumor compartment.

metastases having a diameter of 1.9 cm, even though the latter patient would have a larger tumor burden. Also, the patient with multiple very large metastases would not be distinguished from the patient with a single 2.1-cm nodule if one uses the Samuels' schema. Perhaps in patients with metastases a summing of tumor area or volume will provide the most useful prognostic indicator. This is presently under study by W. DeWys.[4]

At present the M system shown in figure 4 may provide the most meaningful classification for surgical decision making while the Samuels' system provides a tool for expressing response to chemotherapy.

The subdivisions of disease within each stage may be incorporated into the rubric as follows.

Example 1 The testicular tumor is limited to the testes, six nodes are positive on microscopic study of retroperitoneal nodes, markers become negative after node dissection, and all studies for distant metastases are negative.

SII (T_1N_{1b})

Example 2 The testicular tumor has extended along the spermatic cord and the cut end is involved on microscopic study; at the time of retroperitoneal node dissection most of the disease is resected but gross residual tumor remains in the renal hilum; no evidence of distant metastases.

SII (T_2N_4)

Example 3 The testicular tumor is limited to the testes, and enlarged nodes are visualized on lymphangiogram and CT scan. At surgery, enlarged nodes (> 2 cm) are present but all are resected and histologic study shows involvement of eight nodes but no extracapsular extension; postoperatively the βHCG falls slightly and then begins to rise.

CII → SII (T_1N_{2b}) → βHCG pos → CIII ($T_1, N_{2b}M_{1a}$)

[4]DeWys WD. Effect of tumor bulk on response to combination chemotherapy of testicular cancer. In preparation.

N_0 Lymph nodes free of metastases on microscopic examination

N_1 Lymph nodes not visibly enlarged at surgery, but microscopic disease found on pathologic examination

N_{1a} $\leq$ 5 nodes positive

N_{1b} > 5 nodes positive

N_2 Lymph nodes enlarged and microscopically contain tumor but no extension beyond lymph nodes into areolar tissue

N_{2a} Largest nodes $\leq$ 2 cm and $\leq$ 5 nodes involved (both criteria)

N_{2b} Largest nodes > 2 cm and/or > 5 nodes involved

N_3 Extension from lymph nodes into adjacent areolar tissue (microscopic or gross); no gross residual tumor remaining after surgery

N_4 Tumor present in retroperitoneal lymph nodes, and/or retroperitoneal areolar tissue with gross residual tumor remaining after surgery or massive inoperable involvement

FIGURE 3.—Staging of the regional lymph node compartment.

TABLE 2.—Effect of lymph node status on recurrence and survival in nonseminomatous testicular cancer based on Batata (footnote 3)

Category*	Definition	Recurrence†	Survival (5-yr)†
N_0	Microscopic negative	72/248 (29%)	188/248 (76%)
N_1	Microscopic positive	19/52 (37%)	35/52 (67%)
$N_{2,3}$‡	Gross metastases	103/136 (76%)	38/136 (28%)
§	Palpable metastases	17/18 (94%)	2/18 (11%)

*Data of Batata et al have been re-arranged using the definitions in fig 3.

†No. of patients with recurrence or surviving/total No. studied.

‡Batata did not subdivide gross metastatic involvement as to presence or absence of extranodal extension as is recommended in fig 3.

§This category generally, but not precisely, would correspond with category N_4 of fig 3.

M_0 No evidence of distant metastases

M_{1a} Evidence of occult metastases based on biochemical markers and/or other tests

M_{1b} Single metastases in single organ site

M_{1c} Multiple metastases in single organ site

M_{1d} Metastases in multiple organ sites

FIGURE 4.—Staging of the extent of distant metastases.

In conclusion, we have presented a staging schema for testicular cancer encompassing the concepts of a clinical staging system and a surgical staging system. Within the surgical staging system, we have proposed a breakdown of the T and N categories which has biologic and prognostic relevance. In the M category, we concur with the use of the UICC-AJC classification but consider that this classification requires refinement to better express the bulkiness of metastases. We recommend that the utility of this proposed system be tested in clinical trials.

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Minimal Single-Agent Activity of Maytansine in Refractory Breast Cancer: A Southwest Oncology Group Study[1,2]

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SUMMARY

Maytansine is an experimental antitumor agent that has shown minimal efficacy against breast cancer with minimal myelosuppression in phase I trials. Forty-one patients with advanced drug-resistant breast cancer were treated with a 5-day intermittent iv infusion of maytansine repeated every 21 days. All patients had been heavily pretreated with cyclophosphamide, methotrexate, 5-fluorouracil, or doxorubicin, and 12 had received vinblastine, mitomycin C, or investigational drugs. All patients had measurable disease and an expected survival of 6 weeks. The average performance status was 2.5. Twelve patients did not complete one full cycle of therapy, leaving 29 evaluable for response. One patient had a partial regression of pulmonary disease, seven had transient responses of < 50% reduction in tumor or stable disease, and 21 had progressive disease. Toxic effects (vomiting, diarrhea, ileus, lethargy, and altered mentation) were considerable. Since maytansine is a relatively nonmyelotoxic metaphase inhibitor, we feel that even minimal efficacy in heavily pretreated patients justifies further evaluation of the agent in combination therapy.

[Cancer Treat Rep 64:675–677, 1980]

Maytansine is an experimental antitumor agent isolated from an East African shrub (1). Antitumor effect is largely related to metaphase arrest (2), although some inhibition of RNA and protein synthesis does occur. Inhibition of spindle formation in sea urchin eggs by maytansine is 100-fold that of vincristine (3). Activity of maytansine against murine P388 leukemia, B16 melanocarcinoma, and Lewis lung tumors led to phase I trials in human subjects, where a suggestion of efficacy in advanced breast cancer was seen (4,5). There was a suggestion of schedule dependency in the P388 animal model, and clinical response in breast cancer was seen only with 3- or 5-day schedules. The Southwest Oncology Group began a trial in May 1978 to evaluate the efficacy of maytansine in the treatment of drug-resistant breast cancer using a 5-day schedule of administration.

MATERIALS AND METHODS

Forty-one patients with advanced breast cancer were entered in this study from May 1978 to July 1979. One patient was lost to followup. Characteristics of the 40 evaluable patients are listed in table 1. Most patients were older and had multiple sites of parenchymal metastases. All patients were debilitated by disease, and 20 were confined to bed or chair. Twelve patients had received prior vincristine therapy. Twelve patients had elevation of liver enzymes at initiation of therapy. Maytansine was initially given to eight patients at a dose of 0.5 mg/m²/day for 5 days by slow iv push repeated

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TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total	40
Postmenopausal	34
Performance status	
Mean: 2.5	
Median: 3.0	
Age (yrs)	
Mean: 56.9	
Median: 59	
Previous treatment	
Radiation therapy	31
Hormonal therapy	33
Cyclophosphamide, methotrexate, 5-fluorouracil	39
Doxorubicin	39
Vincristine	12
Other chemotherapeutic agents	12
Disease sites	
Lung or pleura	33
Skin, nodes, or breast	29
Bone	24
Liver	18
Brain	8
Multiple sites	34

every 21 days. The starting dose was subsequently decreased to 0.3 mg/m²/day due to toxicity. Six patients initially received a 50% reduced dose due to hepatic dysfunction defined as an SGOT > 60 mg/dl, an SGPT > 50 mg/dl, or a bilirubin level > 2.0 mg/dl. Tumor measurements were obtained prior to each course of therapy in most patients and before at least every other treatment in all patients. Eleven patients were evaluable only for toxic effects: eight due to early death and three due to insufficient data. Twenty-nine patients had tumor measurements repeated at least 3 weeks after their first treatment and were evaluable for response. Twenty-five patients received two or more courses and 15 had an escalation in dose. A complete response is defined as the disappearance of all lesions for ≥ 4 weeks, a partial response is defined as a decrease of ≥ 50% in the size of all measurable lesions for ≥ 4 weeks, stable disease is defined as no significant change for 8 weeks, and increasing disease is defined as the appearance of a new lesion or the increased size of an old lesion.

RESULTS

One patient was considered to have a partial response, with a 50% reduction in pulmonary lesions for 7 weeks while her bone scan remained stable. Six patients had lesser degrees of tumor reduction for < 4 weeks and have been classified as having no response. Of these six patients five had improvement in soft tissue tumors and one with tumor-compromised bone marrow had normalization of her blood cell counts although a bone marrow examination was not repeated. Another patient with stable disease for 9 months continues to receive maytansine. Twenty-one of the 29 evaluable patients had increasing disease.

Forty patients were evaluable for toxic effects. Eighteen patients had no significant toxic effects, while the remaining 22 had considerable toxic effects which necessitated treatment or dose reduction. Toxic effects consisted primarily of vomiting (nine patients), ileus or diarrhea (11 patients), and lethargy (14 patients) followed by confusion or psychosis (seven of the 14 patients). Four patients developed thrombocytopenia and five developed leukopenia. Nine patients died within 3 weeks of therapy initiation. Three of these women had terminal vomiting, ileus, and lethargy and confusion that were possibly related to the drug, although two had known brain metastases. All patients had hepatic metastases but only one had impaired hepatic function. Tumor rather than drug was the probable cause of death in the other six patients. There was no apparent relationship between dose and toxicity. Nine of 15 patients treated with at least 0.5 mg/m²/day of maytansine and 13 of 25 treated with smaller doses had significant toxic effects.

DISCUSSION

Interpretation of new-agent efficacy trials in breast cancer must be tempered by the knowledge that the patient populations studied are heavily pretreated, often preterminal, and unlikely to respond to any therapy. Despite this caveat, we must conclude that maytansine has minimal, if any, efficacy as a single agent for the treatment of advanced breast cancer at a dose and schedule causing considerable toxicity. These results are similar to those of Cabanillas et al (6) who noted one partial response and one minimal response in 18 patients with breast cancer treated with 3-day courses of maytansine. We do not feel that this study should preclude further evaluation of maytansine in combination therapy for breast cancer, since the agent has minimal myelotoxicity, is a potent metaphase inhibitor, and has shown some minimal single-agent effect in heavily pretreated patients.

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Treatment of Acute Leukemia During Pregnancy: Presentation of Nine Cases[1]

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SUMMARY

The hematologic and obstetric problems of nine pregnant patients with acute leukemia are analyzed. Seven patients received chemotherapy during various trimesters of pregnancy, four during the first 10 weeks, and there was no evidence of congenital malformations in any of their offspring. Six of nine babies are alive and healthy with a normal growth 2 months to 15 years after birth, one child was stillborn, and two children died 21 and 90 days after birth. These results were compared with others in the literature and the conclusions are that pregnancy is not a contraindication for treatment of acute leukemia, that cytotoxic drugs may not cause congenital malformations, and that the management of pregnant patients with acute leukemia should be carefully planned.

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The combination of acute leukemia and pregnancy is very infrequent. Virchow described it for the first time in 1845, and since then about 300 cases have been reported (1). In 1964, Moloney (2) reviewed the articles that had been published and noted the lack of a well-defined method for the treatment of acute leukemia during pregnancy. Some investigators preferred not to use cytotoxic agents for fear of harming the fetus (3,4); others felt that they should be used only during the second and third trimesters, when there is less risk of congenital malformation (2,5,6); and the remaining investigators felt that acute leukemia should be treated in the usual manner, in spite of the pregnancy (7–16). At the present time, new chemotherapeutic agents are being used which prolong the life of the patients and thereby increase the chances for gestation. The harmful effects of these new drugs on the embryo or fetus, however, are not well-known. Therefore, we believed it would be interesting to analyze our experience in the management of nine patients with acute leukemia, who were also pregnant. This is one of the largest single series of individuals with these combined conditions.

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PATIENT ANALYSIS

Nine patients who were pregnant and who had acute leukemia were seen during the period from 1963 to 1979. Two were primigravidas and seven were multigravidas; their ages ranged between 18 and 37 years (table 1).

The morphologic diagnosis of acute leukemia was made by examination of the bone marrow by at least two observers. Histochemical staining (PAS, Sudan black, and peroxidase) was also done for six patients (Nos. 4–9) and the results were compatible with the morphologic diagnosis. Six cases were of the lymphoblastic type and three were myeloblastic. The diagnosis of acute leukemia was made during pregnancy in six of the nine patients; the disease had already been diagnosed in the other three. Two of those three patients were in complete remission, and one was in partial remission (table 1).

The pregnancy ran its course without obstetric problems in all nine patients. Chemotherapy with various drugs, at different doses, was administered at different stages of pregnancy (table 2). All patients experienced the following toxic effects, with

TABLE 1.—Acute leukemia and pregnancy: hematologic and obstetric aspects of 9 patients

Patient No.	Age (yrs)	No. of previous pregnancies	Morphologic type of acute leukemia	Time of diagnosis of acute leukemia with relation to pregnancy
1	25	5	Lymphoblastic	5 mos of pregnancy
2	28	1	Lymphoblastic	29 mos before pregnancy
3	24	3	Lymphoblastic	11 mos before pregnancy
4	28	2	Myeloblastic	9 mos before pregnancy
5	27	1	Myeloblastic	During birth
6	18	0	Lymphoblastic	2½ mos of pregnancy
7	37	12	Lymphoblastic	5 mos of pregnancy
8	24	0	Lymphoblastic	3 mos of pregnancy
9	27	5	Myeloblastic	7 mos of pregnancy

TABLE 2.—Acute leukemia and pregnancy: treatment of patients

Patient No.	Trimester of pregnancy	Medication* (total doses)	Status of the acute leukemia at childbirth
1	2nd, 3rd	TR (250 mg) 6-MP (5950 mg)	Complete remission
2	1st, 3rd	6-MP (15,800 mg) MTX (725 mg) CY (3150 mg)	Complete remission
3	1st, 2nd, 3rd	VCR (48 mg) MTX (1000 mg) CY (25,000 mg) 6-MP (18,300 mg) PDN (7600 mg) ARA-C (3500 mg)	Partial remission
4		†	Active
5		†	Active
6	1st, 2nd, 3rd	ARA-C (1600 mg) PDN (3800 mg) 6-MP (250 mg) MTX (600 mg) VCR (24 mg) CY (5000 mg)	Active
7	2nd, 3rd	ARA-C (1400 mg) PDN (3000 mg) 6-MP (4000 mg) MTX (600 mg) VCR (16 mg)	Complete remission
8	1st, 2nd, 3rd	VCR (16 mg) PDN (3000 mg) A (80 mg) MTX (150 mg) 6-MP (1000 mg)	Partial remission
9	3rd	ARA-C (1200 mg) PDN (150 mg) VCR (2 mg)	Partial remission

*TR = triamcinolone, PDN = prednisone, CY = cyclophosphamide, 6-MP = 6-mercaptopurine, ARA-C = cytosine arabinoside, VCR = vincristine, MTX = methotrexate, A = doxorubicin.
†Patient received no chemotherapy during pregnancy.

TABLE 3.—Acute leukemia and pregnancy: perinatal aspects

Infant No.	Gestation period (wks)	Type of birth	Infant characteristics		
			Sex	Weight (g)	Condition
1	38	Spontaneous	Female	2800	Normal
2	38	Induced	Male	3000	Normal
3	40	Spontaneous	Female	2300	Normal
4	34	Spontaneous*	Male	2200	Stillborn
5	41	Cesarean	Female	2900	Normal
6	34	Cesarean	Male	1000	Pancytopenic
7	38	Spontaneous	Female	2400	Normal
8	33	Cesarean	Female	1900	Normal
9	38	Spontaneous	Female	3000	Normal

*Disseminated intravascular coagulation in the mother.

various degrees of severity: myelodepression, alopecia, and nausea and vomiting. Two patients (Nos. 3 and 7) had paresthesia which was attributed to vincristine. However, it was never considered necessary to suspend or modify the chemotherapeutic program. Four patients (Nos. 3, 4, 6, and 8) had infections or hemorrhages which were not severe and were considered to be due to the myelodepression. At the time of birth, three patients were in complete remission, three were in partial remission, and three demonstrated leukemic activity (table 2).

For six patients, birth of the child was by vaginal delivery. In three patients, childbirth was by cesarean section: one because of prolonged labor, one because of danger to the fetus, and one because of premature rupture of the membrane. The gestation period was from 33 to 41 weeks; in six cases, birth was considered to have occurred at term. Except for one patient (No. 4), there were no complications due to infection or hemorrhage in either the spontaneous deliveries or the cesarean sections. This patient developed disseminated intravascular coagulation 24 hours before childbirth. She had not received chemotherapy during her pregnancy, and at the moment of delivery showed leukemic activity, with 90% blast cells. She was treated with heparin, platelet-rich plasma, and packed rbcs with good results, since there were neither hemorrhagic nor thrombotic complications.

Eight babies were delivered live, and one was stillborn. The stillborn child was that of the patient with intravascular coagulation, and death was attributed to acute hypoxia, since the placenta demonstrated leukemic infiltration and multiple thrombi (table 3). Seven babies were of subnormal weight; six of the newborns were female. The literature mentions a predominance of the female sex in these cases (17,18). None of the newborns showed apparent congenital malformations. Chromosome studies were done in four cases (Nos. 6–9) and these were normal. In six cases (Nos. 3 and 5–9) measurements of hemoglobin, hematocrit, and wbc count were done, and a peripheral blood smear and the umbilical cord were examined. The results of these tests and examinations were normal, except for the child of patient 6, who showed pancytopenia.

The progress of the mothers and their children can be seen in table 4. Six babies survived, and are apparently healthy from 3 months to 15 years after birth. Two babies died 21 and 90 days after birth as a result of septicemia and gastroenteritis, respectively. Autopsy of the latter child did not show any evidence of acute leukemia. All of the mothers died from 1 month to 3 years after childbirth because of leukemic activity or intercurrent septic processes. Autopsies of four patients (Nos. 3 and 5–7) showed findings consistent with leukemia or infection.

DISCUSSION

For a long time there has been apprehension about the use of cytotoxic drugs in pregnant women with acute leukemia because of the possible teratogenic effects. The first medication used under these circumstances was 6-mercaptopurine. Moloney (2) studied 21 pregnant women treated with this drug and found no teratogenic effects in any of their children. There was fetal mortality in five of the 21 cases. This figure is lower than that found in a group of 26 patients who received only corticosteroids, where there were ten neonatal deaths.

Other cytotoxic drugs have since been used. These include cyclophosphamide (12), methotrexate (6,13), cytosine arabinoside (11–13,16), vincristine (9,11), and doxorubicin (13). These drugs have not produced defects in the fetuses studied.

TABLE 4.—Acute leukemia and pregnancy: progress of the patients and their children

Case No.	Mother	Child
1	Died 2 mos after childbirth	Alive, 15 yrs
2	Died 3 yrs after childbirth	Alive, 7 yrs
3	Died 16 mos after childbirth	Alive, 6 yrs
4	Died 11 mos after childbirth	Neonatal death
5	Died 1 mo after childbirth	Alive, 4 yrs
6	Died 5 mos after childbirth	Died from septicemia at 21 days of age
7	Died 5 mos after childbirth	Died from gastroenteritis at 90 days of age
8	Died 2 mos after childbirth	Alive, 16 mos
9	Died 2 mos after childbirth	Alive, 2 mos

We have found neither teratogenic effects nor chromosome alterations in the offspring we studied, even though very high doses of cytotoxic agents were reached in all patients and were administered during the first trimester of pregnancy in four (table 2). No toxic effects could be demonstrated in seven of the eight live babies; it could not be determined if the pancytopenia in the other infant was secondary to the medication or attributable to other causes.

Cytosine arabinoside was used in the treatment of one patient (No. 6), and doxorubicin was used in another (No. 8); both drugs were given during the first 3 months of pregnancy. These drugs had been used in only one other case (13) with results similar to those in this report. The mortality rate observed in our nine infants was 21%, which was similar to that noted in reports by other investigators (2, 9,18). This rate is lower than the mortality rate for patients who were not treated during pregnancy: 60%, as reported by Yahia et al (18). Lilleyman et al (12), Krueger et al (11), and Nicholson (14) consider that pregnancy at the time of acute leukemia diagnosis should not influence the administration of chemotherapy in the usual doses.

This concept is confirmed by our observations that the natural course of the acute leukemia was not altered by the pregnancy, and that the babies of the seven patients who received antileukemic therapy did not show congenital malformations; only one died of septicemia. On the other hand, one of the two patients who did not receive chemotherapy (No. 4) had an increase in tumor activity with infiltration of the placenta, disseminated intravascular coagulation, and intrauterine death of the baby. It may be concluded on the basis of reports in the literature and the findings of this investigation that the evolution of the acute leukemia does not change during pregnancy, nor is the pregnancy itself an indication for modifications in the antileukemic treatment since this therapy does not appear to induce fetal malformations even when it is administered in the first trimester. On the contrary, antileukemic treatment must be administered because the risk of the death of the patient and the fetus is greater if it is not. In view of these studies, the practice of therapeutic abortion cannot be justified in every case of this type.

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Single-Agent and Combination Chemotherapy for Extensive Non-Small Cell Carcinomas of the Lung[1]

Stephen Davis,[2,*] Mahesh R. Pandya,[2] and Pietro Rambotti[2,3]

SUMMARY

One hundred and twenty-four patients with extensive bronchogenic carcinoma were randomized to one of three chemotherapeutic regimens. Forty-one patients received cyclophosphamide (600 mg/m² every 3 weeks) (group 1); 47 patients received cyclophosphamide (600 mg/m² every 3 weeks) and CCNU (70 mg/m² every 6 weeks) (group 2); and 36 patients received cyclophosphamide (600 mg/m² every 3 weeks), CCNU (70 mg/m² every 6 weeks), and doxorubicin (40 mg/m² every 3 weeks) (group 3). The objective response rates were 5%, 8%, and 6% in groups 1, 2, and 3, respectively ($P \geq 0.3$). Median survival times were 20.5 months (group 1), 17.8 months (group 2), and 18.8 months (group 3). There was no significant difference in median survival between groups ($P \geq 0.4$). Responders in each group survived longer than nonresponders. Hematologic toxic effects were severe in group 3 and moderate in group 2. Since combination chemotherapy with cyclophosphamide and doxorubicin or cyclophosphamide, doxorubicin, and CCNU is not superior to cyclophosphamide as a single agent and is associated with greater toxicity, these combinations are not recommended as therapy for extensive bronchogenic carcinoma.

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Bronchogenic carcinoma is the leading cause of death by cancer in the US. Since the majority of patients have disseminated disease at the time of diagnosis, effective chemotherapy becomes a major goal of cancer physicians (1).

Single-agent chemotherapy for patients with non-small cell lung cancer has not had significant success. In spite of initial enthusiasm for many single agents (2), larger studies reveal response rates in the range of 5%–15% (3). Combination-chemotherapy regimens have been reported to increase the response rate, ie, survival, in lung cancer. Bitran et al (4) reported a 35% response rate in patients with non-small cell lung carcinoma using cyclophosphamide, doxorubicin (Adriamycin), methotrexate, and procarbazine (CAMP). Livingston (5) reported a 45% response rate using bleomycin, doxorubicin, CCNU, vincristine, and mechlorethamine (nitrogen mustard) (BACON). The same group using cyclophosphamide, vincristine, methotrexate, and bleomycin (COMB) reported that 11 of 23 patients had an objective response rate (6). Chahinian et al (7) reported a response rate of 46% in 41 patients using methotrexate, doxorubicin, cyclophosphamide, and CCNU (MACC); however, patients with small cell histology were also included in the protocol. Despite the enthusiastic response rates in these initial studies the Southwest Oncology Group could not reproduce the response rate with BACON, and the Eastern Cooperative Oncology Group reported a 12% response rate using the MACC regimen (8,9).

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TABLE 1.—Patient characteristics

Characteristic	Group 1	Group 2	Group 3
Mean age in yrs (range)	62 (37–71)	56 (43–73)	57 (41–73)
No. of patients	41	47	36
Histologic diagnosis*			
Squamous cell carcinoma			
11	12	14	11
12	12	10	7
13	2	4	2
Adenocarcinoma			
31	7	7	5
32	4	6	5
Large cell carcinoma			
40	4	6	5
Prior therapy			
Surgery	5	4	2
Radiotherapy	29	34	28
Karnofsky initial performance status			
40–70	29	38	27
80–100	12	9	9

*World Health Organization (1977).

In the hope of clarifying this situation, the present study was designed to further investigate, in one institution, the efficacy of multiple-drug regimens versus single-agent cyclophosphamide in the treatment of extensive non-small cell carcinoma of the lung.

MATERIALS AND METHODS

From July 1976 to January 1978 a total of 124 patients with histologically confirmed non-small cell carcinoma of the lung were entered in the study. There was no selection except that patients were required to have a Karnofsky performance status of > 40 and be classified as having extensive disease (tumor not confined to mediastinum and one hemithorax). Patients had clinically detectable disease measurable either by physical examination of tumor or by roentgenographic techniques. The group consisted of 101 men and 23 women in the age range of 37–73 years (mean, 58). No patient entering the protocol had received prior chemotherapy. Patient characteristics are shown in table 1.

Pretreatment evaluation consisted of history and physical examination, cbc, liver function tests, BUN, creatinine, urinalysis, chest roentgenogram, and radionuclide scanning of liver and bone, and gallium-67 total-body scanning. Response was evaluated every 3 weeks. A complete response was defined as the disappearance of all clinical and radiologic evidence of disease, and a partial response was defined as a decrease of > 50% in the total area for *all* measurable disease, with no evidence of new malignant disease. Patients who had a subjective response, ie, decrease in pain, but no associated decrease in measurable tumor burden, were considered nonresponders. Survival calculations for patients entering this study were evaluated by the two-tailed generalized Wilcoxon-Gehan test (10).

Patients were randomized to receive the following courses of chemotherapy: group 1 received cyclophosphamide at 600 mg/m² iv every 3 weeks; group 2 received cyclophosphamide at 600 mg/m² every 3 weeks and CCNU at 70 mg/m² every 6 weeks; and group 3 received cyclophosphamide at 600 mg/m² iv every 3 weeks, doxorubicin at 40 mg/m² iv every 3 weeks, and CCNU at 70 mg/m² every 6 weeks. Chemotherapy was continued until no further decrease in measurable disease occurred or until obvious progression could be documented. Patients were considered to have had an adequate trial if they were in the study for ≥ 21 days. Planned drug administration was delayed if the wbc count was < 3000/μl or if the platelet count was < 75,000/μl. Patients with this transient leukopenia or thrombocytopenia were then followed weekly and treated as soon as their counts rose above these values.

RESULTS

Therapeutic Benefits

The results obtained in the 124 patients randomized to this protocol are shown in table 2. The objective response rates were 5%, 8%, and 6% in

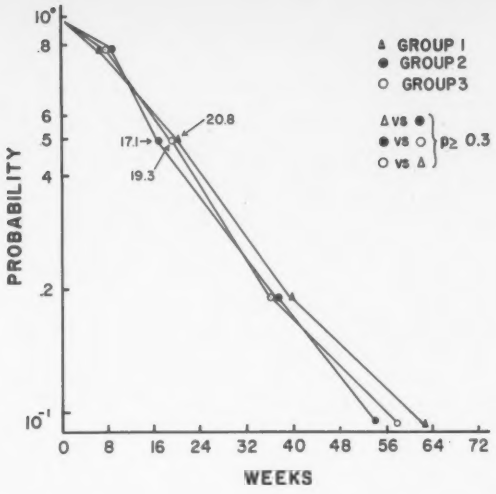

FIGURE 1.—Chemotherapy for extensive non-small cell carcinoma of the lung: survival from start of treatment. Group 1, cyclophosphamide (600 mg/m² every 3 wks); group 2, cyclophosphamide (600 mg/m² every 3 wks) and CCNU (70 mg/m² every 6 wks); group 3, cyclophosphamide (600 mg/m²) and doxorubicin (40 mg/m²), both given every 3 wks, and CCNU (70 mg/m² every 6 wks).

groups 1, 2, and 3, respectively. No patient in any group had a complete response. Regardless of the regimen used, only eight of the 124 evaluable patients had a partial response. All eight patients who responded were ambulatory (initial performance status, 80–100).

The survival rates for *all* patients in each group are shown in figure 1. Median survival time was similar for all three groups: 20.8 weeks (group 1), 17.1 weeks (group 2), and 19.3 weeks (group 3). Median survival for responders compared to nonresponders for each group is shown in table 3. In all groups median survival for responders was greater than that for nonresponders, although significance was only reached in group 2.

Toxic Effects

The toxic effects of the chemotherapeutic protocols are summarized in table 4. The degree of thrombocytopenia and neutropenia was pronounced in group 3 and moderate in group 2. Bleeding was noted in four of 47 patients (10%) in group 3. No transfusions were required in group 1. Infection, as documented by positive bacterial cultures, was seen in eight patients (36%) and in five patients (12%) in groups 3 and 2, respectively. There were no treatment-related deaths seen in any group of patients; however, two patients in group 3 required leukocyte transfusion.

DISCUSSION

The results of this randomized study comparing single-agent cyclophosphamide, cyclophosphamide and CCNU, and cyclophosphamide, doxorubicin, and CCNU show a response rate of 5%, 8%, and 6%, respectively. Therefore, it is obvious that multiple-agent chemotherapy is comparable to single-agent cyclophosphamide therapy for extensive non-small cell lung cancer. In addition, the multimodal regimens were associated with severe and life-threatening hematologic toxicity.

Median survival for all groups was similar whether patients were treated with cyclophosphamide alone, cyclophosphamide and CCNU, or cyclophosphamide, doxorubicin, and CCNU. Similarly, responders had a tendency toward a greater median survival than nonresponders; however, even in the multimodal therapy groups no survival advantage for responders was demonstrated when compared to cyclophosphamide-treated groups. The results are in accord with the data published by Cohen and Perevodchikova (11). These investigators reported on chemotherapy trials involving > 850 patients; the response rate varied between 0 and 12%.

TABLE 2.—Response rate of patients with extensive non-small cell lung carcinoma

	No. of patients		
	Group 1	Group 2	Group 3
Complete responders	0	0	0
Partial responders	2	4	2
Nonresponders	39	43	34
Total (%)	41 (5)	47 (8)	36 (6)

($P \geq 0.3$ for all group comparisons)

TABLE 3.—Comparison of the median survival in responders and nonresponders

	Median survival in wks for—		
	Group 1	Group 2	Group 3
Responders	38.1	40.2	35.6
Nonresponders	15,7	14.9	17.9
All	20.8	17.1	19.3
	(P = 0.22)	(P = 0.05)	(P = 0.18)

TABLE 4.—Hematologic toxic effects

	No. of patients		
Patient data	Group 1	Group 2	Group 3
Wbc count (cells/μl)			
< 1000	0	2	7
1000–2000	0	7	11
2000–3000	17	23	15
> 3000	24	15	3
Platelet count (cells/μl)			
< 20,000	0	0	6
20,000–50,000	7	21	24
> 50,000	34	26	6
Rbc transfusions*	0	4	5
Infection†	2	5	8
Wbc transfusions*	0	0	2

*Chemotherapy-related.
†Culture-positive blood, sputum, spinal fluid, and/or urine.

Since the multiple-drug regimens used in this protocol were not superior to cyclophosphamide alone and are highly myelosuppressive, cyclophosphamide alone is preferable to these combinations in chemotherapy for extensive non-small cell bronchogenic carcinoma.

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Results of the Royal Marsden Hospital Second Soft Tissue Sarcoma Schedule (STS II) Chemotherapy Regimen in the Management of Advanced Sarcoma[1]

Brief Reports and Preliminary Communications

Bruce M. Bryant* and Eve Wiltshaw[2]

SUMMARY

Forty-eight patients with advanced soft tissue sarcoma were treated with a five-drug combination regimen consisting of vincristine, actinomycin D, and cyclophosphamide (Day 1) followed by doxorubicin and an infusion of methotrexate (Day 22). Thirty-seven of the 48 patients were fully evaluable. Of the 37 evaluable patients, two achieved a complete remission and three achieved a partial remission, with an overall response rate of 13.5%. These results compare unfavorably with previously reported series from this and other centers, and the regimen has now been superseded.

[Cancer Treat Rep 64:689–692, 1980]

In August 1975 The Royal Marsden Hospital Sarcoma Unit formulated the second Soft Tissue Sarcoma Schedule (STS II) of combined chemotherapy for the treatment of advanced disease. The treatment scheme consisted of vincristine at a dose of 1.4 mg/m² (maximum, 2 mg) iv, actinomycin D at a dose of 1.4 mg/m² (maximum, 2 mg) iv, and cyclophosphamide at a dose of 600 mg/m² (maximum, 1 g) iv, on Day 1, followed on Day 22 by doxorubicin (Adriamycin) at a dose of 60 mg/m² iv and methotrexate at a dose of 50 mg/m² by iv infusion over 24 hours with 15 mg of folinic acid rescue six times hourly for 36 hours. The regimen was repeated after a 3-week interval and continued, depending on response, for a total of nine courses bringing the cumulative doxorubicin dose to 540 mg/m².

This schedule resulted from the experience of three previous schemes of treatment shown by the Sarcoma Unit to have activity in the management of soft tissue sarcoma (1). The first of these was a single-agent scheme using methotrexate either as an oral or parenteral preparation; the second was a five-drug combination (MDS) incorporating cyclophosphamide, vincristine, actinomycin D, 5-fluorouracil and methotrexate; and the third was a three-drug regimen which combined doxorubicin, DTIC, and a methotrexate infusion. The reason for an alternating regimen was to effectively double the total period of exposure to doxorubicin before the dose limit was reached, and also possibly to overcome development of drug resistance. DTIC was excluded from STS II because of previous patient intolerance to STS I and the lack of improved remission rate of STS I over MDS or methotrexate alone.

PATIENTS AND METHODS

All patients requiring systemic therapy for disseminated or locally advanced nonresectable soft tissue sarcoma were considered. Previous treatment did not exclude a patient from study unless there had been prior chemotherapy with similar drug combinations. The distribution of histologic tumor types is shown in table 1.

Patients were considered to be unevaluable if they withdrew from treatment before two courses had been completed, if they were lost to followup during this time, or if they died during the first course of treatment. Thirty-seven of the original 48 patients were evaluable.

Response was considered complete (CR) if all evidence of disease disappeared for a period of 3 months as judged by clinical examination, roentgenograms, ultrasound scan, and scintiscan where appropriate; response was considered partial (PR) when a $> 50\%$ decrease in all measurable tumor was observed for > 4 weeks. Any patient with $< 50\%$ regression of tumor was regarded as a nonresponder. Patients received a minimum of two treatment courses unless there was rapid progres-

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TABLE 1.—Histologic distribution of tumors

Tumor type	No. of patients	
	Total	Evaluable
Leiomyosarcoma	16	12
Fibrosarcoma	6	5
Malignant fibrous histiocytoma	6	4
Neurofibrosarcoma	4	2
Liposarcoma	3	2
Undifferentiated sarcoma	3	2
Synovial cell sarcoma	2	2
Endometrial stromal sarcoma	2	2
Epithelioid sarcoma	2	2
Rhabdomyosarcoma (pleomorphic)	1	1
Clear cell sarcoma of tendons/aponeuroses	1	1
Malignant giant cell tumor of soft parts	1	1
Malignant fibrous xanthoma	1	1
Total	48	37

sion of disease after a single course. If the latter occurred, the treatment was changed. The patients who responded continued to receive the regimen until relapse or until nine courses were given.

RESULTS

Of the 37 patients, two had CRs and three had PRs (CR rate, 5.4%; overall response rate, 13.5%). The responders are described in table 2.

Patient 1 achieved a CR after 18 weeks of treatment, sustained the CR for only an additional 15 weeks, had relapse at the primary site, and died without further treatment 13 months after start of chemotherapy. Patient 2 also achieved a CR after 18 weeks of treatment, had relapse in the lung 12 weeks after completing a full nine courses, and had thoracotomy and removal of a solitary lung deposit. She is now well and apparently tumor-free 43 months after diagnosis. Both of these patients became symptom-free after treatment, with improved appetites and increased weights.

Two of the three patients who achieved a PR had initial symptoms of pain, with loss of weight and appetite, and both became symptom-free after treatment. Patient 4 remains alive and is responding to alternative chemotherapy. Patient 3 died 9 months after withdrawing from treatment during continuing tumor regression, and patient 5 died shortly after relapsing while receiving treatment. Median survival for responders was 24 months from diagnosis and 13 months from start of STS II and for nonresponders the corresponding figures were 14 and 6 months.

TABLE 2.—Complete and partial responders

Patient No.	Age (yrs)	Sex	Histology	Site	Extent of disease	Response	Duration of response (mos)	Survival from diagnosis (mos)
1	59	F	Endometrial stromal sarcoma	Uterus	Local recurrence only	CR	8	22
2	53	F	Leiomyosarcoma	Uterus	Local recurrence, ?liver deposits, single lung deposit	CR	16.5	43
3	68	M	Synovial cell sarcoma	Ankle	Lung deposits after amputation of foot	PR	Continued response up to 5 mos, then refused further treatment	24
4	31	F	Malignant fibrous histiocytoma	Ovary	Massive local disease, lung and liver deposits	PR	7	30+
5	64	F	Endometrial stromal sarcoma	Uterus	Local recurrence, liver deposits	PR	7	20

TABLE 3.—Performance status of 37 evaluable patients

Status*	No. of complete responders	No. of partial responders	Total No. of patients
100	—	—	—
90	—	—	4
80	—	—	12
70	2	1	10
60	—	1	5
50	—	—	2
40	—	1	1
30	—	—	3
20	—	—	—
10	—	—	—
Total	2	3	37

*Karnofsky scale.

DISCUSSION

Reported response rates to chemotherapy for advanced sarcoma vary widely, even for the same regimen (2,3). Methotrexate (1,4), actinomycin D, and cyclophosphamide are all active in childhood embryonal rhabdomyosarcoma (6–9), and they, together with doxorubicin (5), have significant activity both as single agents (10–12) and as constituents of numerous combinations. It is therefore disappointing and a little surprising that the response rates were so low in this STS II study. The toxicity of the regimen was also low, in that no adverse effects on the kidneys, liver, heart, or mucosal surfaces were observed. Alopecia occurred in all patients. Nausea and vomiting were troublesome, particularly with the VAC part of the regimen, and was presumably due to the relatively high dose of actinomycin D. Hematologic toxic effects were acceptable with no patient requiring reduction in dosage of any of the agents and $< 10\%$ of patient courses requiring postponement due to a total wbc count of $< 3000/mm^3$ or a platelet count of $< 100{,}000/mm^3$.

The reasons for the low response rates are not clear. A generally low performance status at the beginning of treatment would not appear to be the cause, since most of the patients had a Karnofsky performance status of ≥ 70 (table 3). Part of the explanation may lie in the alternating nature of the scheme, which may cause either a simple "dilution" of the tumoricidal effect or possibly a separation of two or more agents which have a synergistic effect on the tumor cell, eg, doxorubicin and cyclophosphamide. It might be suggested that the VAC part of the regimen played little part in the responses and that the regressions were due solely to the doxorubicin and methotrexate combination, but in all of those patients who showed a response, there was invariably an objective reduction in tumor size following the first injection of VAC. It is noteworthy that all but one of the responders were female with genital tract tumors predominantly localized in the pelvis, and this serves to confirm our previous impression that disease at the primary site responds better than metastatic disease (1).

It is clear that the response rates in this study are unacceptably low and therefore this regimen has been superseded. Because of its poor patient acceptability, actinomycin D, at least at this dosage, will not be featured in future schemes. The principle of alternation also requires closer scrutiny, and a search for new active agents will be made for use in sarcoma therapy in the Unit.

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Phase II Evaluation of Vindesine in Patients With Advanced Breast Cancer[1,2]

Brief Reports and Preliminary Communications

Violante E. Currie,* Fernando Camacho, Robert Wittes, and Charles Young[3]

Vindesine is a chemically derived structural analog of vinblastine with antineoplastic activity similar to that of vincristine (1,2) against tumors in rodents. Phase I human studies have shown that a weekly dose of vindesine at 3–4 mg/m² by iv bolus is well-tolerated and suitable for use in phase II studies (2–4). Vindesine is reported to have antitumor activity in patients with breast carcinomas (5), acute leukemias (6), Hodgkin's and non-Hodgkin's lymphomas (6,7), testicular tumors (8,9), and non-small cell carcinomas of the lung (8,10). Vincristine and vinblastine have therapeutic activity in breast cancer (11,12), and have been utilized in combination regimens (13). This present report will relate the results of a phase II trial in patients with advanced breast cancer who have received prior chemotherapy or hormonal therapy.

PATIENTS AND METHODS

Patient Selection

Twenty-five evaluable patients with histologically proven metastatic breast carcinoma were studied between July 1975 and February 1979. All but one of these patients had received multiple-drug chemotherapy prior to the vindesine trial (table 1). All patients were postmenopausal, either by physiologic menopause, surgery, or chemotherapeutic induction. All patients had visceral and/or soft tissue metastases; in addition, 40% of the patients had osseous metastases. All patients had progressive metastatic disease not responsive to conventional cytotoxic chemotherapeutic agents or hormonal therapy, and all had a wbc count of $\geq 4000/mm^3$, a platelet count of $\geq 150{,}000/mm^3$, a BUN level of < 25 mg/dl, and a bilirubin level of ≤ 1 mg/dl.

Pretreatment evaluation consisted of cbc and platelet count determinations, chest roentgenograms, assessments of serum calcium, liver and renal function, and CEA. Isotope scans, additional radiographic studies, and other studies were done when indicated.

Treatment Schedule

Vindesine (supplied by Eli Lilly and Co, Indianapolis, IN) was given at an initial dose of 4 mg/m² to good-risk patients ≤ 60 years old with normal liver and marrow functions and at a dose of 3 mg/m² to marginal-risk patients (3). This dose was increased or decreased by 1 mg weekly as toxicity permitted.

Criteria for Response

An adequate trial was defined as vindesine given at a minimum total dose of 9 mg/m² (ie, three doses). Categories of response are defined as: *partial remission (PR)*—a > 50% reduction in the sum of the products of the largest perpendicular diameter of measurable lesions without progression in any lesion or the appearance of any new lesion for at least 1 month; *minor regression (MR)*—an objective (≥ 25%) reduction in the sum of the products of the diameters of measured lesions but less than required for PR; *stabilization*—a < 25% change in tumor size; and *progression*—an objective increase in measurable disease or the appearance of new tumor lesions.

RESULTS

Therapeutic Effects

One of the 25 patients experienced a PR lasting 15 months. This patient had cutaneous metastases.

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[2]Supported in part by Public Health Service grant CA-05826 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. F. Camacho is a Medical Oncology Fellow.

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TABLE 1.—Characteristics and results of therapy in 25 evaluable women with advanced breast cancer

Characteristic	
Median age in yrs (range)	59 (42–73)
Median Karnofsky performance status (range)	70% (40%–90%)
No. of patients	
Prior cytotoxic chemotherapy	
Cyclophosphamide	24
Methotrexate	20
5-Fluorouracil	23
Doxorubicin	20
Vincristine	1
Vinblastine	3
cis-Dichlorodiammineplatinum(II)	2
Sites of known disease	
Bone	10
Lung	10
Liver	2
Soft tissue/skin/node	22
Central nervous system	1
Sites of measurable disease	
Lung	10
Liver	2
Soft tissue/skin/node	22
No. of doses of vindesine given	
3	2
4	4
5	5
6	14
Toxic effect from treatment	
Stomatitis	1
Paresthesia	10
Jaw pain	2
Constipation	6
Weakness/fatigue	4
Leukopenia ($\leq 2.9 \times 10^3$ cells/mm^3)	14
Thrombocytopenia	1
Response to therapy	
PR	1
MR	6
No change/progression	18

Of the 25 patients six (24%) had MRs and 18 (72%) showed either no change in their disease status or progression of disease. The median time to progression for the entire group was 2 months (range, 4 weeks–15 months).

Toxicity

Vindesine was well-tolerated by the majority of patients at a dose of 3–4 mg/m^2 weekly. Leukopenia ($\leq 2.9 \times 10^3$ cells/mm^3) occurred in 56% of patients; the median leukocyte count nadir was 2.5×10^3/mm^3 (range, 0.9–2.9×10^3). This occurred within 7 days of the last dose of vindesine and was transient; no patient had septicemia. Thrombocytopenia ($< 100{,}000$ cells/mm^3) occurred in only one patient. Peripheral paresthesias were evident in ten patients (36%). Jaw pain and constipation were rare. Alopecia occurred in 100% of patients who were at risk.

DISCUSSION

Although vindesine produced objective antitumor effect in seven of 18 patients, the magnitude of the effect made it clinically useful (ie, PR) in only one woman. These observations suggest that vindesine will not be an effective drug in heavily pretreated patients with advanced breast cancer. These data supplement the observations of Smith et al (5) who obtained only one useful remission in ten patients with breast cancer who had received previous cytotoxic therapy. They noted, however, five useful remissions (complete remission + PR) in ten patients with breast cancer who received vindesine as initial cytotoxic chemotherapy. The 15-month PR in the present study occurred in the only patient in the series who had not received cytotoxic chemotherapy prior to entering the vindesine trial.

It appears possible that vindesine may be a clinically useful agent in patients with breast cancer who have had minimal cytotoxic therapy. Further phase II evaluation of the drug may be desirable following failure of hormonal therapy or as initial therapy in selected patients with estrogen receptor-negative tumors. If further study confirms the drug activity, prospective comparison with vincristine, or the addition of vindesine to combination chemotherapy regimens for treatment of breast cancer, might be appropriate.

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A Phase II Study of Fluorodopan in the Treatment of Advanced Colorectal Cancer[1]

Brief Reports and Preliminary Communications

Kesavan G. Nair, Houshang Moayeri,* and Arnold Mittelman[2,3]

SUMMARY

In an early phase II study, fluorodopan (5-(2′-fluoroethyl-2′-chloroethyl)-amino-6-methyl uracil) was given to 17 patients with advanced colorectal cancer previously treated with multiple agents. Side effects included mild nausea and vomiting in 14 patients, platelet counts of < 50,000/μl in four patients, and wbc counts of < 2000/μl in two patients. Partial remission lasting 9 weeks was achieved in one patient, and stable disease with a mean duration of 7.1 weeks was achieved in ten patients. Given in this setting fluorodopan does not appear to have a significant effect in terms of regression of tumor or prolongation of survival.

[Cancer Treat Rep 64:697–699, 1980]

Single-agent chemotherapy, as well as combination chemotherapy, has been disappointing in the treatment of colorectal cancer. Although response rates from 8% to 80% have been noted in previous reports, survival has not been significantly affected. Efforts of many gastroenterologic oncology programs have shifted to investigation of new single agents and drug combinations (1). At the Roswell Park Memorial Institute we evaluated the efficacy of fluorodopan in the treatment of colorectal cancer.

Fluorodopan, a derivative of uracil mustard, is an alkylating agent that possesses activity like that of an antimetabolite (2). The parent compound, uracil mustard, has shown antitumor activity against a number of transplantable tumors in rodents (3,4), and against non-Hodgkin's lymphoma, Hodgkin's disease, chronic leukemias, and some solid tumors including ovarian cancer in human subjects (5–8). Dopan (5-di(2-chloroethyl)amino-6-methyl uracil) has also shown activity against tumor systems in animal studies and against human neoplasms. On a weight-by-weight basis, fluorodopan was found to be less toxic than, but equally effective as, dopan in animal screens. Neither had cumulative toxicity in mice, but fluorodopan was tolerated in doses 50 times higher than dopan. Central nervous system toxicity, a side effect occurring in the beagle dog at relatively low doses, does not occur in other experimental animals or in man.

In clinical studies uracil mustard was found to have effects comparable to mechlorethamine against chronic leukemias, lymphomas, and Hodgkin's disease (7,8). In studies in the USSR, responses were demonstrated in lymphoma, fibrosarcoma, Kaposi's sarcoma, and nasopharyngeal carcinoma. In a phase I study of fluorodopan at the Roswell Park Memorial Institute, a dose of 60 mg/m² given orally once weekly was found to be effective and well-tolerated.[4] Colorectal cancer was one of the cancers found to respond to fluorodopan.

MATERIALS AND METHODS

Twenty-three patients, 12 males and 11 females, were treated with fluorodopan. All had colorectal cancer with distant metastases, all were previously treated with 5-fluorouracil and methyl-CCNU, nine had received DTIC, eight had received vincristine, three had received melphalan, one had received β-2-deoxythioguanosine, one had received hydroxyurea, and two had previously received radiotherapy to local areas in addition to chemotherapy (table 1). The mean age was 62.9 years (range, 52–77). Informed consent for the use of the drug was obtained. The patients were stratified according to their performance status (PS) at the start of treatment (Eastern Cooperative Oncology Group scale) as follows: *PS 0*—normal activity; *PS 1*—symptomatic but ambulatory; *PS 2*—in bed < 50% of time;

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[3]The authors acknowledge the help of Patricia Burke, RN, in patient followup and Saras Nair, MD, in the translation of the Russian paper.

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[4]Kaufman J, and Mittelman A. Unpublished data.

TABLE 1.—Prior therapy of 17 evaluable patients

Therapy	No. of patients
5-Fluorouracil	17
Methyl-CCNU	17
DTIC	9
Vincristine	8
Melphalan	3
Hydroxyurea	1
β-2-Deoxythioguanosine	1
Palliative radiation	2

PS 3—in bed > 50% of time; and *PS 4*—100% bedridden.

Fluorodopan was given orally at a dose level of 60 mg/m² once weekly and was later modified to lower dose levels depending on wbc and platelet count nadirs. Patients were evaluated for response 4 weeks after initiation of treatment. Response to treatment was determined in terms of complete response, partial response, stable disease, and disease progression. Complete response was defined as the complete disappearance of all demonstrable disease; partial response was defined as a ≥ 50% reduction in the sum of the products of the longest perpendicular diameters of discrete measurable disease with no demonstrable disease progression elsewhere; stable disease was defined as no change in the size of any lesion or a < 25% reduction in size of measurable disease as defined above; and disease progression was defined as a > 25% increase in the products of the longest perpendicular diameters or any measurable lesion.

Cbcs were done weekly and blood chemistries were done every 2–3 weeks. Fluorodopan was given in 5- and 20-mg capsules.

RESULTS

Six of the 23 patients were not evaluable and were therefore excluded from the study for the following reasons: one patient was lost to followup, three patients had no subsequent measurements taken, and two patients received insufficient treatment. Of the remaining 17 patients, three had a PS of 0, nine had a PS of 1, and five had a PS of 2. The results of treatment were as follows. There were no complete remissions. One patient achieved partial remission for a period of 9 weeks, remained stable for a further 2 weeks, then had disease progression. Ten patients had stable disease for a mean duration of 7.1 weeks. The remaining six patients had disease progression while they were receiving fluorodopan. Primary disease indicators were: abdominal tumor, six patients; lung, five patients; liver, four patients; rectal, one patient; and sacral tumor, one patient.

Toxicity

All but three patients experienced moderate nausea and vomiting. In one patient nausea and vomiting were sufficiently intense to warrant a reduction in dosage. Table 2 summarizes hematologic toxicity. Thrombocytopenia (platelet count < 100,000/μl) and leukopenia (wbc count < 4000/μl) occurred in nine and 11 patients, respectively, but after dose modification, remained within an acceptable range. Severe toxicity, with platelet counts < 50,000/μl and wbc counts < 2000/μl, was encountered in four patients (24%) and in two patients (12%), respectively. Other systemic toxic effects were not encountered. The average duration of treatment was 8.6 weeks. The mean total dose given was 1430 mg/patient.

DISCUSSION

The objective of the present phase II study was to evaluate antitumor activity of fluorodopan in the treatment of advanced colorectal cancer. During the phase I study of this agent, 33 evaluable patients received different doses from 5 to 100 mg/m². Among 13 patients who received < 60 mg/m², leukopenia (wbc count < 4000/μl) and thrombocytopenia (platelet count < 100,000/μl) were seen in three patients. All patients had received prior therapy and 11 had received prior methyl-CCNU. Among 20 patients who received ≥ 60 mg/m², leukopenia was observed in 12 patients and thrombocytopenia was observed in 11. All patients had received prior therapy and 17 had received prior methyl-CCNU. Since the prior therapy, including methyl-CCNU, was comparable in the two groups, increased myelotoxic effects in patients who received larger doses of fluorodopan were thought to be primarily due to fluorodopan. Accordingly, the dose with acceptable toxicity was felt to be about 60 mg/m² once weekly for phase II study. Utilizing the dose and schedule in this study, fluorodopan was found to be a reasonably well-tolerated drug.

TABLE 2.—Hematologic toxicity of fluorodopan

Nadir	No. of patients
Wbc count (cells/μl)	
<2000	2
2000–4000	9
>4000	6
Platelet count (cells/μl)	
<50,000	4
50,000–100,000	5
>100,000	8

All but one patient tolerated the drug well without serious gastrointestinal toxicity, and that patient was able to tolerate the drug at a lower dose. Myelotoxicity was encountered in a significant number of patients, but on reduction of the dose, it was possible to continue use of the drug. One patient achieved partial remission for 9 weeks. In ten patients (59%) the disease progression was arrested, with a mean duration of 7.1 weeks for stable disease. In the remaining six patients, the disease progressed despite treatment. In this study, fluorodopan contributed insignificantly to the survival of patients with advanced cancer of the colon and the rectum. However, it was given to patients who had progressive disease after failure while receiving other agents, and the results of the study thus cannot exclude possible beneficial effect if the drug is given as a primary agent before tumor sensitivity is altered.

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Pulmonary Function Tests During Adjuvant Lung Irradiation for Osteogenic Sarcoma[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Six patients received whole-lung irradiation at a dose of 2000 rads/4 weeks (nominal standard dose = 700 Rets) after surgery for primary osteogenic sarcoma. Pulmonary function tests performed before and 6–20 months after pulmonary irradiation failed to demonstrate any pulmonary function impairment in any of the patients studied.

[Cancer Treat Rep 64:701–703, 1980]

Radiation therapy to the thorax produces variable changes in pulmonary function (1) and radiographic appearance (2). Early manifestations of these changes are characterized by infiltration, and later manifestations by fibrosis. These changes depend on the irradiation field, dose and fractionation, time from completion of irradiation, and tissue response to irradiation.

Adjuvant whole-lung irradiation has been used for the treatment of osteogenic sarcoma (3,4) and for therapy for metastatic testicular carcinoma (5) at doses of 2000–2500 rads given in 3–4 weeks, with the suggestion that these doses rarely cause radiation pneumonitis or subsequent fibrosis; however, no results of early and late pulmonary function tests were reported in these studies. During our osteogenic sarcoma studies concerning the value of adjuvant whole-lung irradiation with or without subsequent doxorubicin (Adriamycin) treatment (6), we were interested in evaluating the early and late changes in pulmonary function and radiographic appearance secondary to irradiation and/or doxorubicin.

MATERIALS AND METHODS

Six of 18 consecutive patients with osteogenic sarcoma of the bone seen at the Instituto Nacional de Enfermedades Neoplasicas between September 1975 and October 1976 volunteered for pulmonary function evaluation before and 6–20 months after treatment. All patients received 2000 rads of whole-lung irradiation from a cobalt source, administered in 21–28 days of treatment immediately after surgical resection of primary tumor (100–133 rads/fraction, nominal standard dose [NSD] = 669–750 Rets). Starting 4 weeks after pulmonary irradiation, four of six patients received doxorubicin at a dose of 20 mg/m² iv for 3 days repeated every 6 weeks to a total dose of 475–550 mg/m². All six patients had amputation or hemipelvectomy as their primary treatment and then had followup examinations every 4–6 weeks including chest roentgenogram, cbc, and renal and liver function tests.

The baseline and followup pulmonary function studies were performed with a Wedge spirometer (Med-Science Electronics) for pulmonary volume and capacity. Nitrogen was used to determine the residual volume. Alveolar-arterial gradient for CO_2 was measured with a Gobart capnograph and a Beckman analyzer for O_2 in the alveolar air; arterial blood obtained simultaneously was measured with a Radiometer electrode. CO diffusion was measured with an Instrumentation Associates analyzer. Recording was made in an 8-channel Gilson registry.

RESULTS

Table 1 summarizes the clinical aspects of each one of the six patients studied. There were two

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TABLE 1.—Clinical summary of patients studied

Patient No.	Sex	Age (yrs)	Radiation therapy (NSD = Rets/date)	Doxorubicin dose/date	Disease recurrence site/date	Palliative therapy	Symptoms/date	Survival (mo)/type	Pulmonary function test done after pulmonary radiotherapy at—
1	M	21	750/10–8–75	475 mg/6–20–77	Lung/6–26–77	Followup	—	27/died	6 mos
2	F	13	700/11–28–75	None	Lung/6–12–78	Followup	Dyspnea/8–25–78	38/died	8 mos
3	M	18	685/2–16–76	None	Lung/5–18–77	Pulmonary lobectomy/6–7–77	Hemoptysis/7–18–77	36/NED†	6 and 19 mos
4	F	25	674/1–5–76	500 mg/5–13–77	Lung/11–26–76	Pulmonary lobectomy/1–18–77	Dyspnea/11–14–77	38/NED	6 and 21 mos
5	M	20	669/1–9–76	550 mg/3–23–77	—	—	Dyspnea/5–6–77	38/NED	6 and 20 mos
6	M	21	669/4–12–76	510 mg/7–22–77	—	—	—	36/NED	18 mos

*Baseline pulmonary function test also done for all patients.
†NED = no evidence of disease.

females and four males. One patient was 13 years of age, while the others were between 18 and 25 years old. There was no major difference in the number of Rets of whole-lung irradiation received by each patient; the dose ranged from 669 to 750 Rets. Four of six patients (Nos. 2–5) had cardiorespiratory symptoms, three patients (Nos. 2, 4, and 5) had dyspnea, and one patient (No. 3) had hemoptysis > 16 months after lung irradiation. Three of these patients (Nos. 2–4) had symptoms concomitant with recurrent metastatic disease in the lung and/or after lobectomy, patient 5 developed transitory symptoms of dyspnea 2 months after completing doxorubicin therapy to a dose of 550 mg/m^2. Chest roentgenograms did not show any evidence of radiation pneumonitis or fibrosis. Baseline pulmonary functions were obtained in all patients before whole-lung irradiation, in five patients ≥ 6 months following completion of irradiation, and in four patients repeated ≥ 18 months following completion of irradiation.

Table 2 summarizes the mechanical, distribution, blood flow, diffusion pulmonary function, blood gases, and gradient results in the patients studied. The changes observed in the above parameters did not exceed the normal expected range for each determination, although in patients 3 and 4 there is diminution of the VC, FRC, and D_LCO in the 19- and 21-month study as compared to their baseline and 6-month pulmonary function studies; there is no difference in the mean values of the groups.

DISCUSSION

Radiation pneumonitis and later radiation fibrosis may occur between doses of 3000 and 3500 rads and it is more frequently seen at doses > 4000 rads; however, human variability in the pulmonary response to radiation is an unmeasurable factor (2). Moreover, in circumstances where radiation is used in the lung for therapeutic reasons (usually in doses > 4000 rads), acute radiation pneumonitis which markedly deteriorates the pulmonary function is reversible by therapy, with often-limited interstitial fibrosis leading mostly to the reduction of ventilation. The persistent respiratory capacity depends on the functional status of the remaining lung (1). Witek et al (1) report that in patients who have successful irradiation of their lung tumor, improvement of pulmonary function may result from removal of respiratory disturbance with improvement in blood gases and airway resistance. With prophylactic or adjuvant whole-lung irradiation in doses of 2000–2500 rads in 3–4 weeks, radiation pneumonitis and fibrosis were rare (3–5). Wara et al (7),

TABLE 2.—Pulmonary function of patients studied

Measurement*	Test values (means ± SD)		
	Before irradiation (baseline)	6 mos after irradiation	20 mos after irradiation
VC (liters)	3.73 ± 0.69	3.88 ± 0.87	3.57 ± 1.00
FRC (liters)	2.85 ± 0.56	2.77 ± 0.55	2.80 ± 0.99
MVV (liters/min)	108 ± 42.36	106 ± 31.91	126.50 ± 25.04
FEV_1 (%)	91 ± 5.73	86 ± 3.71	88.0 ± 2.00
EV(BTPS) liters/min	7.42 ± 1.21	7.33 ± 0.88	7.09 ± 1.61
AV(BTPS) liters/min	4.28 ± 0.85	4.35 ± 0.63	4.33 ± 0.93
N_2 washout	1.50 ± 0.25	1.22 ± 0.25	1.26 ± 0.23
D_LCO	20.35 ± 5.47	17.93 ± 4.31	21.45 ± 6.71
CO Capt (%)	49.05 ± 6.29	51.54 ± 2.24	53.72 ± 2.59
Pa O_2	10.233 ± 4.18	103.60 ± 6.27	110.75 ± 3.30
PA CO_2	36.67 ± 1.87	36.40 ± 3.29	37.25 ± 2.22
PA O_2	93.83 ± 4.92	97.00 ± 4.06	101.75 ± 2.06
Pa CO_2	39.83 ± 2.14	41.20 ± 3.03	39.25 ± 4.19
pH	7.38 ± 0.01	7.38 ± 0.03	7.37 ± 0.03
HCO_3	23.02 ± 1.07	23.90 ± 1.66	22.50 ± 2.04
B. Ex	−1.25 ± 0.94	−0.84 ± 1.05	−1.70 ± 1.17
A-aO_2	8.63 ± 3.26	6.60 ± 5.46	9.00 ± 4.08
A-aCO_2	3.17 ± 2.86	4.60 ± 1.78	3.00 ± 1.83

*VC = vital capacity; FRC = functional residual capacity; MVV = maximum ventilatory vol; FEV_1 = forced expiration vol in 1 min; EV = external ventilation; AV = alveolar ventilation; N_2 = nitrogen washout; D_LCO = diffusion capacity for CO; CO Capt = caption for CO; PA O_2 = partial pressure of alveolar O_2 (mm Hg); PA CO_2 = partial pressure of alveolar CO_2 (mm Hg); Pa O_2 = partial pressure of arterial O_2 (mm Hg); Pa CO_2 = partial pressure of arterial CO_2 (mm Hg); HCO_3 = bicarbonate (mEq/liter); B. Ex = base excess (mEq/liter); A-aO_2 = alveolar arterial-gradient for O_2 (mm Hg); A-aCO_2 = alveolar arterial-gradient for CO_2 (mm Hg).

in studies concerning therapeutic lung irradiation at different doses and the incidence of pneumonitis, report that whole-lung irradiation in doses of 2500 rads in 20 fractions (NSD = 770 Rets) yields only a ≤ 5% probability of pneumonitis.

Consistent with the clinical experience of others (3–5) and the studies of Wara et al (7), we saw no episodes of radiation pneumonitis or radiation fibrosis in the 18 patients studied. Furthermore, our studies of pulmonary function at 6 and 20 months after 2000 rads of whole-lung irradiation (NSD = 669–750 Rets) do not show any change in the pulmonary function of these patients. The minor changes seen in patients 3 and 4 were most likely associated with pulmonary function changes secondary to the therapeutic lobectomy for recurrent disease in the lungs of these two patients before their 19- and 21-month pulmonary function studies.

Although adjuvant whole-lung irradiation in the treatment of osteogenic sarcoma had no clinical effectiveness in our previous studies (6), we confirm in the present report that whole-lung irradiation at doses of 2000 rads in 21–28 days (669–750 Rets) does not produce adverse effects to the patient (radiation pneumonitis, fibrosis, or changes in the pulmonary functions) in the time these patients were studied.

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Phase II Evaluation of *N*-(Phosphonacetyl)-L-aspartic Acid (PALA) in Patients With Non-Small Cell Carcinoma of the Lung[1,2,3]

Brief Reports and Preliminary Communications

Ephraim S. Casper,* Richard J. Gralla, David P. Kelsen, Alan Houghton, Robert B. Golbey, and Charles W. Young[4,5]

Asparate transcarbamylase catalyzes a key reaction in the pathway of pyrimidine biosynthesis, the condensation of carbamyl phosphate with aspartic acid, generating carbamyl-L-aspartate. *N*-(Phosphonacetyl)-L-aspartic acid (PALA), a stable analog of the transition state in that reaction, is a potent inhibitor of asparate transcarbamylase (1). PALA has demonstrated antitumor activity in both Lewis lung carcinoma and B16 melanoma, two solid tumor models resistant to other antimetabolites (2). Phase I studies have shown that PALA given at a weekly dose of 4.5 g/m^2 is nonmyelosuppressive and produces moderate, but manageable, gastrointestinal and cutaneous toxicity (3). Most of the drug is excreted unmetabolized in the urine (4,5). Because of its unique site of action, its activity in resistant solid tumor models, and its lack of myelotoxicity, a phase II study of PALA in non-small cell lung cancer was initiated.

MATERIALS AND METHODS

Twenty-two patients with a histologically or cytologically confirmed diagnosis of non-small cell carcinoma of the lung were treated. All patients had objectively measurable lesions which had not been previously irradiated and a performance status of $\geq$ 50% (Karnofsky scale). Normal hepatic and renal functions, a wbc count of $\geq$ 4000/mm^3, and a platelet count of $>$ 100,000/mm^3 were required for entry in the study.

Eighteen patients were evaluable for therapeutic response. The median age of these patients was 58 years (range, 41–75), the median performance status was 70% (range, 50%–90%), and the male:female ratio was 13:5. The evaluable patients included 12 with adenocarcinoma, five with epidermoid carcinoma, and one with large cell anaplastic carcinoma. Five patients had received no previous chemotherapy.

PALA was administered as a 45–60-minute iv infusion. The initial dose was 4.5 g/m^2 weekly for patients with a performance status of $\geq$ 70% and 3.75 g/m^2 weekly for patients with a performance status of 50%–60%. If toxicity was not encountered after 3 weeks of therapy, the dose was escalated by 20%. If severe toxicity occurred, therapy was discontinued until the abnormality was resolved, and treatment was resumed at a dose that was 20% lower than the initial dose.

An adequate therapy trial was defined as treatment consisting of three doses of PALA with at least moderate toxicity, or two doses of the drug with the development of profuse diarrhea, rash involving $>$ 25% of the skin surface, and unequivocal progression of disease. Therapeutic responses were defined as: *complete remission*—the disappearance of all evidence of disease for at least 4 weeks; *partial remission*—a $\geq$ 50% reduction in the sum of the products of the two greatest perpendicular diameters of all measurable lesions for at least 1 month with stabilization or improvement of performance status; and *minor response*— a $>$ 25% reduction of all measurable lesions, but less than that required for a partial remission, or a $>$ 50% reduction of measurable lesions in the presence of clinical deterioration.

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[3]PALA was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

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RESULTS

Therapeutic Responses

No major objective responses occurred. Of four minor responses observed, three were in patients with adenocarcinoma and one was in a patient with epidermoid carcinoma. The patient with epidermoid carcinoma had a > 50% reduction in the size of a metastatic lymph node; the reduction lasted for 1 month, but the patient experienced clinical deterioration related to drug toxicity. Two of the three patients with adenocarcinoma had minor responses in their primary pulmonary lesions lasting 2 and 6 months, respectively; these were associated with subjective improvement. In a third patient with adenocarcinoma, response was noted in a metastatic supraclavicular lymph node; this response was not of clinical benefit. Two patients had stabilization of disease for 7 and 8 months, respectively.

Toxicity

As anticipated from the phase I studies (3–5), myelosuppression was not seen. Cutaneous toxic effects were prominent, and consisted of an erythematous macular eruption which eventually scaled or ulcerated. This rash typically began on the face, scalp, and intertriginous areas, but occasionally involved the trunk and the arms or legs. Eight patients had mild rash or none at all. In seven patients the rash involved 10%–25% of the body surface area and in three patients it involved 25%–50% of the body surface area. Cutaneous toxicity generally began during the second week of treatment and resolved over 7–10 days.

Gastrointestinal toxic effects occurred in 14 patients. Nine patients experienced mild diarrhea and five patients had uncomfortable diarrhea interfering with their normal activities. Bloody diarrhea or fluid and electrolyte imbalance did not occur as a result of therapy. Three patients developed mild stomatitis. There was no evidence of hepatic or renal toxicity.

Although neurotoxicity was seen in large animal toxicology studies,[6] it was not clearly observed in phase I clinical trials. Two patients in this study, however, developed profound neurologic disturbances. One patient who had previously undergone excision of a solitary cerebral metastasis developed status epilepticus after receiving her second dose of PALA. The seizures could not be controlled and she died. Post-mortem examination revealed the surgical defect, but only a microscopic focus of tumor in the contralateral hemisphere. Another patient received a total of eight doses of PALA but had to have therapy discontinued when he developed mild confusion. One week later he developed right temporal lobe status epilepticus which was partially controlled after several days. A computerized axial tomographic scan and cerebral arteriogram were normal, and the seizures and confusion were resolved over a period of 4–6 weeks.

No etiology other than PALA toxicity could explain the occurrence of status epilepticus in these patients. Similar neurotoxicity has been noted in patients with other diseases who have been treated with PALA;[7] the mechanism is not known.

DISCUSSION

PALA was selected for clinical trials because of its activity in murine solid tumor models and its lack of myelotoxicity. It is of particular interest because of its unique site of biochemical action.

This study included only patients with good performance status, five of whom had received no prior chemotherapy. Although toxic effects were seen in all patients, no major responses were observed. At 95% confidence limits, the predicted true response rate is < 16%.

The development of uncontrollable seizures in two patients was an unexpected occurrence. Patients who are to receive this drug in other studies should be informed of this possible toxic effect and patients with known structural CNS disease should be excluded from further studies using PALA until the nature of the neurotoxicity is more clearly defined.

We conclude that when given at this dose on a weekly schedule, PALA is not effective as a single agent in the treatment of non-small cell carcinoma of the lung.

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cis-Dichlorodiammineplatinum(II) in the Treatment of Esophageal Carcinoma[1]

Brief Reports and Preliminary Communications

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SUMMARY

Seventeen consecutive patients with untreated squamous cell carcinoma of the esophagus were treated with *cis*-dichlorodiammineplatinum(II) (DDP) by infusion at a dose of 3 mg/kg with mannitol diuresis. One patient had a partial response and six additional patients had subjective improvement in dysphagia and associated weight gain; however, no complete responses were noted. Toxic effects were mainly renal. There were no drug-related deaths. We conclude that DDP alone is not effective in squamous cell carcinoma of the esophagus.

[Cancer Treat Rep 64:709–711, 1980]

Despite advances in surgical and radiologic techniques in the treatment of squamous cell carcinoma of the esophagus, the prognosis for patients remains poor. Five-year survival in all patients with squamous cell carcinoma of the esophagus is < 5% regardless of whether therapeutic radiation or surgery is used as the primary modality of therapy (1). Chemotherapy for advanced carcinoma of the esophagus is at present unsatisfactory. Only bleomycin has been reported to be effective, with response rates ranging from 0 to 60%; in cases where a response has been noted, the duration has been very short (2,3)

cis-Dichlorodiammineplatinum(II) (DDP) has shown antitumor activity against bladder carcinoma, testicular carcinoma, and squamous cell carcinomas of the head and neck (4). Together with bleomycin, DDP has also been reported to be effective in the treatment of squamous cell carcinoma of the esophagus. Kelsen et al (5) reported four partial responses (PRs) in 25 previously untreated patients. To date, however, there has been no report concerning the effectiveness of DDP as a single agent in the treatment of squamous cell carcinoma of the esophagus. Accordingly, we conducted a trial of high-dose DDP in the treatment of this disease. The present report describes our findings.

MATERIALS AND METHODS

Seventeen male patients with histologically confirmed squamous cell carcinoma of the esophagus are the subject of this report. The median age was 62 years (range, 48–79). All patients were to have a Karnofsky performance status of > 60%. Forty-seven percent of the patients were black and 53% were caucasian. No patient had any form of radiotherapy, chemotherapy, or surgery prior to entry in this study. The primary tumor was considered to be located in the upper third of the esophagus in three patients (17%), in the middle third in seven patients (41%), and in the lower third in seven patients (41%) (table 1). All 17 patients had a history of > 20 pack/years of cigarette smoking, 11 of the 17 patients (65%) were considered heavy ingestors of ethanol (> 60 ml/day), and one patient had asbestos exposure. No patient was admitted to the study with a second malignancy, ie, bronchogenic, head and neck. Informed consent was obtained from all patients, and the protocol used was approved by the Human Studies Committee.

Prior to entry in this study, all patients had a history and physical examination, cbc with differential and quantitative platelet count, chest roentgenogram, urinalysis, 24-hour urine specimen for calculation of creatinine clearance, electrolytes, SMA-12, and barium esophagogram. Except electrolytes and SMA-12 which were measured daily, these baseline studies were repeated every 4 weeks just prior to the next course of therapy. Each cycle of DDP was given every 4 weeks in the hospital with close monitoring of blood cell counts, creatinine, and electrolytes. All patients received a min-

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TABLE 1.—Characteristics of 17 male patients and their response to DDP therapy

Characteristic	No. of patients (%)	No. of patients responding
Age (yrs)		
Mean: 62		
Range: 48–79		
Site of primary esophageal tumor		
Upper 3rd	3 (18)	0
Middle 3rd	7 (41)	0
Lower 3rd	7 (41)	1 (PR)

imum of 2000 kcal/day of nutritional support while receiving therapy.

DDP was given by iv infusion over 1 hour at a dose of 3 mg/kg. All patients received 6 hours' hydration with normal saline, and a diuresis maintained with mannitol given over 12 hours as previously described (6). Complete remission was defined as the disappearance of all measurable disease as determined by barium esophagograms. PR was defined as a decrease of > 50% in the sum of the products of the two longest perpendicular diameters of measurable tumors. Patients who had subjective improvement in dysphagia or weight gain were considered nonresponders. Barium esophagograms were evaluated by visual estimation for reduction in tumors by three staff radiologists who had no prior knowledge as to the status of the patient. Chemotherapy was discontinued after two courses if no response was detected.

RESULTS

Among the 17 patients who are the subject of this report, there was one response (a PR) as determined by barium esophagograms every 4 weeks. This patient was given two additional courses of DDP (total, four) with no further reduction in tumor. Having failed initial chemotherapeutic intervention, all patients were then given radiotherapy for their disease. Despite the PR seen in the one patient, his survival was almost identical to the mean survival achieved by the remaining 16 patients, ie, 51 versus 42½ weeks, respectively. These survival data are comparable to those reported in larger series (1). Six patients reported a decrease in dysphagia; all had a weight gain of > 5% of body weight. However, their esophagograms were said to show no change from baseline. Nutritional status did not appear to affect response or survival.

The observation that toxicity in this group of patients was mild is compatible with our previous results at the same dosage schedule of DDP in patients with head and neck carcinoma (6). There were no episodes of sepsis during the minimal leukopenia and no life-threatening hemorrhages secondary to thrombocytopenia.

As in our previous experience using DDP in a single-agent chemotherapeutic regimen for head and neck carcinoma, the main toxic effects of DDP therapy were renal (6). Seven of the 17 patients treated had transient increase in their serum creatinine; only two had increases of > 2 mg/dl. All serum creatinine levels returned to normal after therapy was discontinued. Transient renal tubular dysfunction was documented in three patients. This was manifested as hypomagnesemia in two patients and as hypokalemia in a third. These findings were similar to our previously reported data (7).

DISCUSSION

The results of this study show that, in the regimen described, DDP has minimal if any effectiveness in the treatment of squamous cell carcinoma of the esophagus when used as a single agent. Only one of 17 patients had an objective response. These results are clearly a disappointment, since it was hoped that DDP chemotherapy would improve the rather dismal outlook for patients with esophageal carcinoma.

Kelsen and co-workers (5) reported that DDP and bleomycin caused PRs in four of 25 previously untreated patients with squamous cell carcinoma of the esophagus. In a second group comprising treated and untreated patients, they reported two partial responders and one complete responder in a total patient population of 12. Thus, among 37 patients, they had seven responders (19%). Bleomycin had been reported to induce remission rates in the range of 0–60%. Bonadonna et al (2) reported a response rate of 60% with a median duration of response between 1 and 10 months. On the other hand, Ravry et al (7) did not have a single responder among the 14 patients they studied. Therefore, it is difficult to evaluate the actual role of DDP in Kelsen's group of patients with carcinoma of the esophagus. From our present results and the results previously described for the response rate of bleomycin, it appears likely that DDP exerts a minimal effect in squamous cell carcinoma of the esophagus. If our present data are confirmed, the use of DDP alone in the treatment of this disease would not be warranted.

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Phase II Ftorafur Therapy in Previously Treated Squamous Cell Cancers of the Head and Neck[1,2]

Brief Reports and Preliminary Communications

Mark Campbell and Muhyi Al-Sarraf[3,*]

SUMMARY

A prospective study was carried out to determine the effectiveness and tolerance of ftorafur (2.25 g/m² daily for 5 days, repeated every 4 weeks) in previously treated patients with advanced squamous cell cancers of the head and neck. Seventeen patients were entered in the study; 15 were evaluable for drug toxicity, and 14 were evaluable for response. Three patients had mild nausea, three had mild-to-moderate lethargy, two had nonspecific generalized weakness, and two had severe stomatitis. Of 15 patients three experienced leukopenia (causing death in one) and four had thrombocytopenia (life-threatening to one). Response to treatment included: progressive disease (nine patients), stable disease (four), and complete response (one with no previous radiotherapy or chemotherapy).

[Cancer Treat Rep 64:713–715, 1980]

In patients with squamous cell cancers of the head and neck, single-agent activity has been demonstrated for a number of chemotherapeutic drugs, including methotrexate, cyclophosphamide, hydroxyurea, vinblastine, doxorubicin (Adriamycin), and cisplatin (1–9). Response rates have varied from 0 to 40% and durations of response have been relatively short.

5-Fluorouracil (5-FU) is also an effective agent in the treatment of head and neck cancers, with an average remission rate of 15% (range, 0–33%) (10). In the experience of Wayne State University, six of 19 patients with advanced head and neck tumors (31.5%) responded to 5-FU (five partial responders and one complete responder) for an average of 6.8 months (7,9).

Ftorafur is another pyrimidine nucleoside derivative that is similar to 5-FU. It was initially synthesized in Latvia in 1966 and has undergone extensive investigation in the USSR (11) as well as in Japan (12–14). In human studies, ftorafur has shown activity in cancers of the breast (11,14–16) and gastrointestinal tract (11,14,16), as well as suggestion of activity in ovarian (11), tongue (11), brain (11), and lung (16) cancers.

On a weight basis, ftorafur has less hematologic and gastrointestinal toxicity than bolus administration of 5-FU (11,17,18). In a phase I study at Wayne State University, six patients received 20 courses of ftorafur with dosage ranging from 2.25 to 3.0 g/m² (iv injection for 5 consecutive days repeated every 28 days) without any myelotoxic effects. To our knowledge, ftorafur had not been evaluated in patients with head and neck cancers, and it seemed reasonable to consider the trial reported here as a phase II study.

MATERIALS AND METHODS

Seventeen patients with measurable, advanced, biopsy-confirmed squamous cell carcinoma of the head and neck were entered in a phase II study with ftorafur. Patients' characteristics are outlined in table 1. To be eligible the patient had to have a life expectancy of at least 8 weeks, a performance status of at least limited self care, a wbc count of $\geq$ 4000 cells/mm³, and a platelet count of $\geq$ 100,000 cells/mm³.

Ftorafur was administered as a 2-hour iv infusion, daily for 5 days, at a dose of 2.25 g/m² in 250 ml of 5% glucose in distilled water. Courses were repeated every 28 days provided that the patient had recovered from the toxic effects of the previous course. Weekly cbcs and platelet counts were scheduled.

The dose of ftorafur was decreased by 0.5 g/m² if grade IV hematologic toxicity occurred (wbcs $\leq$ 1000/mm³ and platelets $\leq$ 25,000/mm³). Complete

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TABLE 1.—Patients' characteristics and response to ftorafur therapy

Patient No.	Age (yrs)	Sex	Primary site	Site at treatment	Prior treatment			Performance status (%)	No. of courses	Response
					Surgery	Radiation therapy	Chemo-therapy*			
1	71	M	Uvula	Local	X	X		80	3	SD
2	48	M	Mouth floor	Local		X	M	50	1	PD
3	80	M	Nasopharynx			X	C	80	4	No measurable disease
4	66	M	Larynx	Local	X	X	MOB	90	3	PD
5	53	M	Ear	Local		X	M	50	1½	PD
6	66	M	Pyriform sinus	Lung, brain	X	X	POB-M	50	2	PD
7	56	M	Pyriform sinus	Local	Bx	X	POB-M	70	1	PD
8	67	M	Mouth	Local	X	X	POB-M	80	1	PD
9	58	F	Pyriform sinus	Bone, local, lung	X	X	POB	50	1	Early death
10	53	M	Pyriform sinus and esophagus	Lung	X	X	POB-M	90	5	PD
11	55	M	Epiglottis	Local	X	X	POB-M	70	4	SD
12	56	M	Larynx	Local	X	X	POB-M, MC	80	4	SD
13	59	M	Larynx	Local	X	X	P		1	Early death
14	60	M	Pyriform sinus	Local		X	M		2	SD
15	44	M	Mouth floor	Skin, local	X	X	POB-M	60	1	PD
16	51	M	Mouth floor	Local	X			80	3	CR
17	59	M	Tonsil	Local	X	X	POB-M	80	1	PD

*M = methotrexate, C = cyclophosphamide, O = vincristine (Oncovin), B = bleomycin, P = cisplatin, MC = mitomycin C.

response (CR) is defined as the disappearance of all demonstrable disease; partial response (PR) is defined as a ≥ 50% reduction of measurable lesion, stable disease (SD) is defined as measurable disease less than PR or an increase in diameter less than progressive disease, and progressive disease (PD) is defined as an increase of ≥ 25% of all measurable lesions.

RESULTS

Fifteen of the 17 patients were evaluable for toxic effects. The two inevaluable patients died shortly after the first drug course ("early death"): patient 9 from rapidly advancing CNS disease, on Day 5 after chemotherapy, and patient 13 suddenly from unknown causes (in a nursing home), 16 days after completing chemotherapy. Six other patients died while in the study; four deaths were not felt to be drug-related, whereas two were felt to be related to ftorafur treatment (one patient's death was secondary to severe stomatitis and the other had acute CNS confusion progressing to coma and death). Side effects of ftorafur treatment included mild nausea (three patients), mild-to-moderate lethargy (three), severe stomatitis (two), and nonspecific generalized weakness (two). One patient was noted to have transient elevation in liver function tests (twice the normal values). Three of the 15 evaluable patients experienced neutropenia, all following at least four courses of therapy; in one of these patients it was felt to contribute to death. Four patients experienced thrombocytopenia; one episode was severe and life-threatening.

Fourteen patients were evaluable for response to ftorafur. Nine patients had no response and showed evidence of PD. Four patients had SD at the time of death: after two, three, four, and five courses of therapy. One patient (No. 16), the only patient who was not previously treated with both radiation therapy and chemotherapy, achieved a CR.

DISCUSSION

In a group of 13 patients who had had previous chemotherapy for squamous cell cancer of the head and neck, ftorafur by iv infusion produced SD in four and no response in nine.

Drug toxicity was generally mild but contributed to the death of two patients. Drug-related myelosuppression with ftorafur was generally mild. However, significant myelosuppression was encountered in patients who had received repeated courses, perhaps suggesting a cumulative effect. Previous reports (19) determined that CNS toxicity was dose-related while recent reports suggest that the clearance of ftorafur may be saturated (20). The latter suggestion may explain the acute CNS toxicity seen in our patient following 5 daily repeated

courses and perhaps requires adjustments in scheduling.

Ftorafur produced a CR in the only patient of the series who had had no previous radiation therapy or chemotherapy. In that patient the drug was interrupted for 2 months, allowing reactivation of his disease which again responded to re-treatment with the drug.

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Combination Chemotherapy for Non-Small Cell Bronchogenic Carcinoma With Bleomycin, Doxorubicin, Methotrexate, and *cis*-Dichlorodiammineplatinum(II) (BAMP)[1,2]

Brief Reports and Preliminary Communications

Steven E. Vogl,[3,*] David Wollner,[4] Barry H. Kaplan,[3] and Marc Berenzweig[3,5]

Chemotherapy for non-small cell bronchogenic carcinoma is generally unsatisfactory. Reproducible response rates have generally been reported as < 20% (1). We were led to investigate a combination of bleomycin (BLM), doxorubicin (Adriamycin), methotrexate (MTX), and *cis*-dichlorodiammineplatinum(II) (DDP) (BAMP) in this group of diseases because of an objective response rate of 61% we observed with BLM, MTX, and DDP in the chemotherapy of squamous cell cancer of the head and neck (2). We reasoned that if this three-drug combination has activity in these less common cancers of the upper aerodigestive tract, perhaps similar activity would be seen in the most common squamous cell cancer in the US, that primary in the bronchus. Doxorubicin was added because of its reported efficacy in non-small cell bronchogenic carcinoma (1). It was expected that the four-drug regimen would still be tolerable, since the three-drug combination had produced little myelosuppression early in the patients' course. The regimen was employed in patients with adenocarcinoma and large cell anaplastic carcinoma of the lung, in addition to the epidermoid type, since encouraging evidence of activity had been reported in these cell types with DDP combinations with doxorubicin (3), doxorubicin and cyclophosphamide (4–6), and doxorubicin and ftorafur (7).

MATERIALS AND METHODS

Eligibility was restricted to patients with histologically proven diagnoses of non-small cell bronchogenic carcinoma, beyond hope of cure by surgery or radiation therapy, and tumor nodules that could be serially measured (or at least evaluated) for response. Entry requirements included a serum creatinine < 1.5 mg/dl, a wbc count ≥ 4000/μl, and a platelet count ≥ 100,000/μl. Patients who had heart disease or had had prior chemotherapy with any of the protocol agents were excluded. Patient characteristics are shown in table 1.

Patients were treated with a 21-day cycle: 10 units of BLM im on Days 1, 8, and 15; 40 mg/m² of MTX im on Days 1 and 15; 40 mg/m² of doxorubicin iv on Day 1; and 50 mg/m² of DDP iv on Day 4 with a 2-hour program of 2 liters of hydration and diuresis with furosemide and mannitol (8). Doses of MTX and doxorubicin were reduced 50% for wbc counts of 3000–4000/mm³ or platelet counts of 75,000–100,000/mm³, and all treatment was withheld for cell counts below these levels.

Of the 31 patients who entered the study, five were excluded from final analysis. Of four of these five patients with squamous cell cancer, two received inadequate doses of chemotherapy during the first 6 weeks, one refused further treatment on Day 15 for reasons not related to drug toxicity, and one died of pneumonia 7 days after beginning treatment without evidence of drug toxicity. The single patient with adenocarcinoma excluded from analysis died from an iatrogenic pneumothorax on Day 17, after receiving only a single dose of the drugs. The median age of the 26 evaluable patients was 58 years (range, 42–72). Only three patients were > 65 years old.

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TABLE 1.—Characteristics of 26 evaluable patients

Characteristic	% of patients
Female	35
Performance status (ECOG scale)*	
0	27
1	50
2	12
3	11
Histologic type	
Squamous cell carcinoma	54
Adenocarcinoma	35
Large cell anaplastic carcinoma	11
Prior therapy	
Surgery	31
Radiation to primary	23
Radiation to metastases	15
Chemotherapy	4
Sites of involvement at entry	
Primary and/or nodes	77
Distant lung	23
Bone	19
Brain	15
Skin	15
Nodes	12
Liver	4

*ECOG performance status scale: 0 = asymptomatic; 1 = ambulatory with symptoms; 2 = bedridden < 50% of time; and 3 = bedridden > 50% of time.

RESULTS

Twelve (46%) of the 26 patients evaluated had objective responses. The median duration of response from the time of starting chemotherapy was 4 months, with a median survival for responding patients of 5½ months, compared to a median survival of 4 months for those patients who did not respond. Seven of the 14 patients with squamous cell carcinoma responded, with a median duration of 5 months (range, 2–13). The median survival of responding patients with squamous cell cancer was 9.5 months versus 4.5 months for nonresponders, but the survival curves cross at 10 months. The difference in survival is not significant ($P = 0.7$, log-rank test). Three of nine patients with adenocarcinoma responded for 3, 3, and 5½ months. Two of three patients with large cell anaplastic carcinoma responded for 1 and 4 months.

Toxicity was substantial. There was one treatment-related death from pneumonia occurring during a period of severe myelosuppression. The following cell counts were recorded: *wbcs*—< 1000/mm³ in one patient, 1000–2000/mm³ in six patients, and 2000–3000/mm³ in five patients; *platelets*—25,000–50,000/mm³ in two patients and 50,000–75,000/mm³ in three patients. Three patients required transfusions, and an additional ten patients had hemoglobin levels < 11 g/dl. Renal toxic effects were mild and transient in two patients (highest creatinine was 1.9 mg/dl for 1 week and 4.1 mg/dl for 2 weeks). One patient had life-threatening mucosal toxicity, three had severe stomatitis, and six had stomatitis of moderate severity. No treatment-related heart failure, deafness, or pulmonary fibrosis was encountered.

DISCUSSION

The 50% response rate observed with BAMP in squamous cell cancer in the present study is encouraging. The substantial toxicity of the regimen and its minor effect on survival, even when responders are compared to nonresponders, make us reluctant to recommend it for further use as it is now designed.

It may be that most of the responsibility for the high order of activity noted in squamous cell cancers comes from DDP rather than the other drugs. Cooperative group trials have recently failed to substantiate earlier claims from single institutions for substantial activity of combination regimens containing the other agents included in BAMP: bleomycin plus doxorubicin (plus mustard, vincristine, and CCNU) (1) and methotrexate plus doxorubicin (plus cyclophosphamide and CCNU) (9). DDP has been inadequately studied as a single agent in this group of patients. The only phase II trial of patients not exposed to prior chemotherapy employed only a modest dose of the drug (5), approximately 25% of that maximally tolerated (10). The only other published phase II study involved mostly patients who had had prior chemotherapy, and would thus be expected to have a substantially reduced rate of response (11).

We believe the high response rates observed with BAMP and other DDP combinations, especially in squamous cell cancer (12), strongly justify a phase II trial of intensive DDP in better-risk patients with advanced measurable non-small cell bronchogenic carcinoma who have not failed prior chemotherapy.

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Phase II Study of Maytansine and Chlorozotocin in Patients With Disseminated Malignant Melanoma[1,2]

Brief Reports and Preliminary Communications

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With few exceptions disseminated malignant melanoma remains quite refractory to most of the available cytotoxic chemotherapeutic agents. The most consistent responses have been obtained utilizing DTIC (1) and the several nitrosoureas (2), which resulted in regression rates of 15%–20%. Various combinations of the nitrosoureas (2) with DTIC, vincristine, or cyclophosphamide have not resulted in much, if any, improvement in regression rates. The combination of DTIC with *cis*-dichlorodiammineplatinum(II) and vincristine has been equally ineffective (3).

For the past 8 years our experience with disseminated malignant melanoma has varied only slightly, with minimal regression rates resulting only from the nitrosoureas and DTIC; most of the patients entering clinical trials at the Mayo Clinic have had visceral dominant disease upon entry and have had mean survivals of only 14–16 weeks from the start of treatment.

Two agents deemed reasonable for clinical trial in this patient population were chlorozotocin and maytansine. Both offered the potential advantage of minimal or no myelosuppressive activity; thus, if any clinical activity were demonstrated, combination therapy with previously known active and myelosuppressive agents would seem reasonable.

Maytansine, a naturally occurring macrolide, possesses mitotic inhibitory properties similar to those of the vinca alkaloids.[4] Maytansine has shown activity against a variety of solid tumors of animal origin including B16 melanoma. A phase I study utilizing maytansine has recently been completed at the Mayo Clinic (4), and a tolerable dose of 0.6 mg/m²/day for 3 days was established.

Chlorozotocin is a water-soluble chloroethyl nitrosourea with a cytotoxic group attached to the C-2 position of glucose. It was specifically designed to modify the dose-limiting delayed hematologic toxic effects that limited the clinical value of previously utilized nitrosoureas. Chlorozotocin has curative activity against L1210 leukemia and B16 melanoma at doses which do not depress the absolute neutrophil count in mice. The principal toxic effects observed during the preclinical toxicologic evaluation were renal tubular necrosis and uremia; hematologic toxic effects were not observed in monkeys but were seen in dogs at lethal dose levels. A recently completed phase I trial of chlorozotocin at Georgetown University (6) indicated that single iv doses of 120 mg/m² produced delayed thrombocytopenia in patients who had previously received chemotherapy. No significant leukopenia was observed at doses of 120–150 mg/m²; however, transient delayed hepatic transaminase elevations were observed 4 weeks after chlorozotocin doses of this level. Nausea and vomiting were observed in half of the patients.

The current clinical trial was proposed to assess the two new agents primarily in untreated patients with advanced malignant melanoma, utilizing a crossover design upon failure to the initial drug treatment which was based on random selection.

MATERIALS AND METHODS

Thirty-two patients (18 men and 14 women; mean age, 53 years) with disseminated malignant melanoma were included in this study. None of the patients had previously received chemotherapy with either of the study drugs; five patients had had previous cytotoxic chemotherapy exposure (two who were receiving chlorozotocin and three receiving maytansine during the study). Only one patient, who was receiving maytansine during the study, had had previous CCNU exposure. Four patients had previously received DTIC in one or another

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[4]Helman L, Henney J, and Slavik M. Clinical brochure: Maytansine (NSC-153858). Prepared by the Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, Feb 1976, 45 pp.

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combination program, and one patient had received melphalan 10 years prior to entering this clinical trial. Twenty-five of 32 patients had multiple systems involved with disease, including 20 with pulmonary tumors. Twenty-five patients had visceral dominant disease and seven patients had soft tissue dominant disease at the time of entry in the study. The two groups were balanced with respect to the presence or absence of dire visceral involvement and number of organ sites involved with tumor.

All of the patients were acceptable candidates for cytotoxic chemotherapy with respect to hematologic status. Nineteen patients were virtually asymptomatic while six patients had performance scores of 2 and seven patients had performance scores of 3 (Eastern Cooperative Oncolgy Group scale). All of the patients were randomly allocated to one of the two treatment programs. If failure was demonstrated, the patient received the alternate drug if still a candidate for study. Maytansine was given at a dose of 0.6 mg/m² iv on each of 3 successive days, with evaluation and re-treatment occurring at 4 weeks. Chlorozotocin was given at a dose of 150 mg/m² iv on Day 1, with evaluation and re-treatment every 5 weeks. Objective regression was defined as a decrease by at least 50% in the product of the perpendicular diameters of the indicator lesion(s), lasting ≥ 4 weeks.

RESULTS AND DISCUSSION

In the group exposed to maytansine as primary therapy, no patient achieved initial clinical response (table 1), while five remained stable for a mean duration of 10 weeks. In 11 patients, disease immediately progressed. Of the group who received chlorozotocin as initial treatment, one patient had an objective regression (table 1), and ten patients remained stable for a mean duration of 13 weeks. In five patients, disease immediately progressed. The one objective clinical regression, lasting 8 weeks, was in a patient who had hepatic as well as lymph node metastatic disease.

TABLE 1.—Results of primary and secondary treatment

Treatment	No. of regressions/No. of patients with— Visceral dominant disease	Nonvisceral disease
Primary		
Maytansine	0/12	0/4
Chlorozotocin	1/13	0/3
Secondary		
Maytansine	0/6	0/1
Chlorozotocin	0/8	0/2

TABLE 2.—Toxic effects of primary and secondary treatment

Toxic effects	Maytansine group (No. = 23)	Chlorozotocin group (No. = 26)
Nausea and vomiting	10	17
Diarrhea	10	1
Neurologic effects	7	0
Wbcs of ≤ 3400/mm³	1	4
Platelets of < 100,000/mm³	0	4

Secondary therapy was equally unsuccessful, with none of seven patients responding to maytansine and none of ten responding to chlorozotocin. Two patients in each group remained stable for 8 weeks. In the majority of patients, disease progressed immediately following exposure to the second type of treatment. Median survival of both groups was in the range of 110 days, with time to progression averaging 30 days for maytansine and 70 days for chlorozotocin. The toxicity associated with these programs is shown in table 2. Nausea and vomiting occurred in ten of 23 patients receiving maytansine, and in 17 of 26 patients receiving chlorozotocin. In none of the patients experiencing gastrointestinal toxic effects was the use of parenteral support necessary. Diarrhea, as well as bowel dysfunction similar to that experienced with the vinca alkaloids, occurred more commonly with maytansine. Neurologic toxic effects, seen only with maytansine, were manifested by myalgias and sensory changes. Myelosuppression was distinctly unusual in this clinical trial and was observed in only four of the 26 patients receiving chlorozotocin and one of 23 patients receiving maytansine. Drug doses were not escalated since, in most instances, the patients did not achieve clinical benefit; thus, crossover to alternate drug therapy was accomplished at the doses chosen for this study.

Neither chlorozotocin nor maytansine would seem to offer any therapeutic advantage for patients with disseminated malignant melanoma over any of the commercially available chemotherapeutic agents. This experience is, however, at variance with that of Hoth et al (6) who did report clinical activity in patients with malignant melanoma with the same dose schedule of chlorozotocin. Toxicity was acceptable, in the absence of clinical response, at the doses chosen. Higher drug doses could well have been utilized, particularly with chlorozotocin.

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Photosensitivity Reaction Following DTIC Administration: Report of Two Cases[1]

Brief Reports and Preliminary Communications

Thomas M. Beck,* Nan E. Hart, and Charles E. Smith[2]

DTIC has demonstrated efficacy in the treatment of many malignancies, including melanoma (1,2), grade III and IV astrocytomas (3), Hodgkin's disease, and various sarcomas (4). Reported side effects include nausea and vomiting (usually most severe on the first 1–2 days of administration), thrombocytopenia, elevation of SGOT, occasional elevation of BUN, a flu-like syndrome, dizziness, chills, and malaise (1). This report describes two patients who developed photosensitivity reactions following the administration of DTIC, suggesting this may be a previously unrecognized toxic effect.

CASE REPORTS

Patient 1

Patient 1 was a 16-year-old white male with a 6-month history of grand mal seizures prior to the diagnosis of grade III–IV astrocytoma. Initial therapy consisted of one course of BCNU (150 mg/m^2 iv for 3 days) followed by whole-brain irradiation. Following completion of radiation therapy, he continued to receive BCNU at 6-week intervals.

Prior to the third course of BCNU, he developed nausea, vomiting, and recurrent headaches. A repeat EMI scan revealed an enlarging mass in the area of previous tumor. He was given dexamethasone which decreased his papilledema; he then received DTIC at a dose of 175 mg/m^2 daily for 5 days. His initial therapy was accompanied by severe nausea, poorly controlled with Compazine. He continued receiving DTIC at 3-week intervals with gradual escalation of his dose to 300 mg/m^2 daily for 5 days. Gradual improvement in the headaches was observed. Papilledema gradually resolved. The patient continued receiving dexamethasone, Dilantin, and phenobarbital.

At the beginning of Course 7 of DTIC, while returning home with his right arm protruding from the car window, the patient experienced stinging and burning of the right hand and arm and right side of the face. This was followed by development of diffuse, intense erythema on the sun-exposed areas. The stinging and erythema recurred daily on sun-exposed skin after the patient received DTIC. No vesiculation or urticaria developed. The erythema subsided 3–4 days after completion of a 5-day course of DTIC. The patient found that he was able to prevent the rash by wearing long-sleeved shirts and a wide-brimmed hat and by otherwise limiting sun exposure following DTIC administration. However, any sun exposure during the day following DTIC administration continued to produce erythema and burning at sun-exposed sites. Compazine was discontinued briefly during subsequent courses of DTIC, but was later re-instituted because it did not seem to alter the above-described reaction.

DTIC was discontinued after 12 courses due to recurrent symptoms and recurrent development of papilledema. The patient's disease was progressively worsening and he died 3 months later. During the 3 months between discontinuation of DTIC and his death, he continued receiving Dilantin, dexamethasone, and phenobarbital. Photosensitivity reactions were not subsequently observed, despite continued sun exposure.

Patient 2

Patient 2 was a 65-year-old white woman with a diagnosis of rhabdomyosarcoma of the left thigh. At the time of initial diagnosis, she was found to have bilateral pulmonary metastases. Initial therapy consisted of doxorubicin (Adriamycin) at a dose of 60 mg/m^2 on Day 1 and DTIC at a dose of 250 mg/m^2 on Days 1–5 repeated every 3 weeks. Combid spansules were given in an attempt to control nausea and vomiting. At the time of her first course of doxorubicin-DTIC, she also began a course of radiation therapy to the tumor of her left thigh. Chemotherapy was continued throughout the course of radiation therapy without incident. The chemotherapy was moderately well-tolerated with the exception of modest nausea and vomiting on Days 1 and 2 and anorexia on Days 3–5.

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On the second day of Course 6 of chemotherapy, while riding home in her car, the patient developed a stinging, pruritic, erythematous rash on the right side of her face. This rash recurred daily following administration of DTIC. Beginning with Course 7, Combid spansules were discontinued, however, the burning, erythematous rash again developed following sun exposure. The area of erythema extended over a greater area and included the right neck, face, and upper sternal area. Doxorubicin was omitted from Course 8 and the burning and erythema again developed in sun-exposed areas. The erythema subsided 3–4 days following completion of therapy with DTIC. Vesiculation and urticaria did not develop. As with patient 1, this patient also found she could reduce the occurrence of this rash by wearing long sleeves and a broad-brimmed hat. DTIC was continued for a ninth course. Burning and intense erythema again developed in sun-exposed areas. Following the ninth course of therapy with DTIC, enlargement of the pulmonary metastases was observed. Other chemotherapeutic agents were subsequently administered and use of Combid spansules resumed without the development of a similar photosensitivity reaction.

DISCUSSION

We describe two patients in whom a phototoxic reaction developed immediately following the administration of DTIC. Both patients experienced intense burning followed by erythema on body parts exposed to direct sunlight within 1–2 hours after iv administration of DTIC. Both patients experienced recurrent photosensitivity reactions following repeat administration of DTIC. The burning and erythema were reduced in both instances by protection with clothing. Discontinuation of phenothiazine antiemetics and other possible photosensitizing drugs did not result in substantial alteration of the above-described reaction.

DTIC is known to be activated by exposure to light (5) and we speculate that one or more of the light-activation products resulted in the photosensitivity reactions observed in our patients.

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Activity of the Anthracycline Agent, 7-Con-*O*-methylnogarol (7-OMEN), Administered Orally to Mice Bearing P388 or L1210 Leukemia[1,2]

Brief Reports and Preliminary Communications

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With the technical assistance of Thomas F. deKoning[4]

SUMMARY

The magnitudes of the therapeutic and toxic responses observed when 7-con-*O*-methylnogarol (7-OMEN) was administered orally to mice inoculated ip or iv with P388 of L1210 leukemia were essentially equivalent to those seen after ip administration of the drug, indicating a significant degree of gastrointestinal absorption.

[Cancer Treat Rep 64:727–729, 1980]

7-Con-*O*-methylnogarol (7-OMEN) is an anthracycline compound prepared by chemical modification of the antitumor antibiotic nogalamycin (1). 7-OMEN is active against a wide spectrum of mouse tumors and is 5-12 times less potent than doxorubicin (Adriamycin) (2). Since 7-OMEN is less than one fifteenth as potent as doxorubicin in inducing the characteristic anthracycline cardiomyopathy in rabbits (3), it could have a therapeutic advantage over doxorubicin.

7-OMEN also possesses certain interesting biochemical properties which distinguish it from nogalamycin, doxorubicin, and other anthracycline compounds (4,5). In studies of cell growth and macromolecular synthesis inhibition with L1210 cells in culture, the ID50 (drug concentration required for 50% inhibition compared to controls) for cell growth was 0.3 μM compared to > 46 μM for RNA and DNA synthesis inhibition, suggesting that cytotoxicity is not mediated through nucleic acid synthesis inhibition. Concentrations of 7-OMEN up to 100 μM produced 0 inhibition of highly purified DNA and RNA polymerases. Furthermore, 7-OMEN was found to be weakly bound to DNA (compared to doxorubicin and other nogalamycin analogs) by methods of circular dichroism difference spectrum and DNA thermal denaturation and by the effect of premixing the agents and calf thymus DNA on L1210 leukemia cell growth inhibition. Similar studies with nogalamycin and other analogs showed that RNA synthesis and RNA polymerase activity were inhibited at cytotoxic concentrations and that these agents were strongly bound to DNA. Although recent studies have suggested multiple actions of doxorubicin (6,7), its cytotoxicity is thought to be at least partially mediated through strong binding to DNA resulting in RNA synthesis inhibition (7).

For clinically useful agents, assuming equal safety and efficacy for oral and parenteral routes of administration, the oral route is more convenient for the patient and physician. Doxorubicin is not active when given orally (8).[5] Certain other anthracyclines (carminomycin, 4-demethoxydaunorubicin, 4-demethoxydoxorubicin) were reported to be active after oral administration although at doses several-fold higher than optimum parenteral doses (9).

Modest activity of orally administered 7-OMEN was noted in early testing against P388 mouse leukemia (2). A more extensive investigation of the oral activity of 7-OMEN showed quite significant results which are reported here. Doxorubicin was also tested in some studies for comparative purposes.

MATERIALS AND METHODS

Agents.—7-Con-*O*-methylnogarol was prepared by published methods at The Upjohn Company (1). Doxorubicin was provided by J. D. Douros, Devel-

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[4]We also gratefully acknowledge the help of Julianna C. Stewart and Roberto Garza.

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[5]Screening data summary on Adriamycin (NSC-123127) from R. Brennan, Automated Information Section, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD, May 14, 1979.

opmental Therapeutics Program, National Cancer Institute, Bethesda, MD.

Chemotherapy studies.—Mouse tumor treatment studies were conducted as previously described (2). Male CD2F1 (BALB/c♀ × DBA/2♂) mice were inoculated ip (six mice per group) or iv (ten mice per group) with 10^6 P388 or 10^5 L1210 cells on Day 0, and drugs were administered as solutions in 0.01 M glucuronic acid once daily on Days 1–9 (qd×9). Control animals received oral doses of 0.01 M glucuronic acid. The injection volume was 0.5 ml in all experiments. Oral doses were administered by gavage.

The therapeutic response measured was median survival time, and results were expressed as percent increase in lifespan (% ILS) calculated as (survival time of treated group/survival time of control group)–1 × 100. Toxicity was measured as weight loss between Days 1 and 5 after tumor inoculation and as median lifespan less than controls.

RESULTS AND DISCUSSION

Table 1 shows the activity of orally and ip administered 7-OMEN against ip and iv inoculated P388 and L1210 leukemias. As previously reported (2), ip administered 7-OMEN was quite active against both leukemias inoculated ip. Equivalent activity against the ip tumors was seen when the agent was given orally, indicating that the extent of gastrointestinal absorption was very significant. Additionally, in animals with iv inoculated leukemia, the response to ip and oral 7-OMEN administration was essentialy equivalent. Weight loss (an indication of drug toxicity) was also indicative of efficient absorption. However, the twofold higher optimal doses for orally administered 7-OMEN in most experiments suggested that gastrointestinal absorption was not complete. In the experiments with ip inoculated tumors, 7-OMEN administered orally as a suspension in 4% ethanol plus 0.9% NaCl gave dose-response curves superimposable on those obtained from drug administered as a solution in 0.01 M glucuronic acid (data not presented).

In previous experiments where ip administered doxorubicin and 7-OMEN were compared on the qd×9 schedule, the two agents were about equally effective against ip inoculated P388 leukemia; 7-OMEN appeared to be more active against ip in-

TABLE 1.—Activity of 7-con-*O*-methylnogarol in P388 and L1210 mouse leukemias*·†

P388 leukemia							L1210 leukemia						
				Survival time (days)		%					Survival time (days)		%
Tumor site	Drug route	Dose (mg/kg/day)	Weight change‡	Median	Range	ILS (cures)	Tumor site	Drug route	Dose (mg/kg/day)	Weight change‡	Median	Range	ILS (cures)
Ip	Ip	25	−4.1	9.0	6–10	Toxic	Ip	Ip	25	−3.6	9.0	8–10	13
		12.5	−2.2	17.5	5–30	59 (1/6)			12.5	+0.9	15.5	10–24	94
		6.25	+0.6	22.5	20–25	105			6.25	+0.6	14.5	12–18	81
		3.13	+1.1	18.0	17–19	64			3.13	+2.1	11.0	11–12	38
	Oral	50	−4.6	10.0	9–11	−9		Oral	50	−4.0	8.5	7–9	6
		25	−2.6	19.5	13–26	77			25	−1.7	16.5	15–18	106
		12.5	+0.1	17.0	16–19	55			12.5	+1.2	12.0	11–15	50
		6.25	+0.9	13.0	9–15	18			6.25	1.4	9.0	9–10	13
		3.13	+1.6	10.5	10–14	−5			3.13	..2	8.5	8–11	6
		Control	+1.8	11.0	10–15	—			Control	2.2	8.0	7–9	—
Iv	Ip	25	−3.9	8.0	7–10	0	Iv	Ip	25	-3.8	9.0	6–10	50
		12.5	−2.1	14.0	10–22	75			12.5	−2.0	13.0	12–15	117
		6.25	+0.3	13.0	12–15	65			6.25	−0.4	9.0	8–10	50
		3.13	+0.8	10.0	9–11	25			3.13	+0.2	7.0	7–8	17
	Oral	25	−2.4	11.5	9–13	44		Oral	25	−2.5	12.0	9–15	100·
		12.5	−0.5	17.0	14–19	113			12.5	−0.7	11.0	6–13	83
		6.25	+0.2	11.5	10–13	44			6.25	−2.0	8.0	8–9	33
		3.13	+0.6	9.0	8–10	13			3.13	+0.4	7.0	6–7	17
		Control	+1.2	8.0	7–9	—			Control	+1.0	6.0	6–7	—

*Doxorubicin was tested concurrently against both the iv inoculated P388 and L1210 leukemias and was inactive and nontoxic to 8 mg/kg/day, orally; when given ip against iv P388, a 38% ILS was obtained at the optimal dose of 2 mg/kg/day; when given ip against iv L1210, a 33% ILS was obtained at the optimal dose of 4 mg/kg/day.

†Agents were administered as solutions in 0.01 M glucuronic acid once daily for 9 consecutive days starting on Day 1 after tumor inoculation.

‡g/mouse between Day 1 and Day 5.

oculated L1210 leukemia on the qd × 9 schedule (2). In the studies reported here, doxorubicin as previously observed (8), was not effective when given orally; 7-OMEN was more effective than doxorubicin when given ip to mice with iv inoculated leukemias (2).

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LETTERS

Anaphylaxis Due to Oral Melphalan

The common side effects of melphalan include nausea, vomiting, leukopenia, and thrombocytopenia. Rarer toxic reactions such as pulmonary fibrosis and acute leukemia due to prolonged use of oral melphalan have been reported. We report here a patient who developed an anaphylactic reaction while receiving oral melphalan adjuvant chemotherapy for breast cancer.

CASE REPORT

A 57-year-old white female noticed a right axillary painless swelling in September 1977. Biopsy showed lymph nodes with metastatic breast carcinoma. Bilateral xeromammogram revealed tumor lesions in both breasts and suspicious lymph nodes in the left axilla. Preoperative bone and liver scans, chest roentgenogram, electrocardiogram, blood cell counts, and liver and renal functions were normal. After biopsy of the left breast lesion showed adenocarcinoma, the patient had a bilateral modified radical mastectomy. She had a past medical history of allergy to penicillin, sulpha drugs, and codeine. She was taking Dyazide (one capsule twice daily) for mild hypertension, lorazepam (1 mg daily) for nervousness, and aspirin occasionally for pain. The patient was seen in The Milton S. Hershey Medical Center Oncology Clinic in November 1977. Results of a physical examination were normal, with well-healed bilateral mastectomy scars. She was advised to start to receive radiotherapy to the chest wall. She also began to receive adjuvant chemotherapy with melphalan at a dose of 0.15 mg/kg orally daily for 5 days, repeated every 6 weeks for 2 years. She completed four courses of melphalan with no side effects. Twenty-four hours after taking the last dose of melphalan in the fifth course, she developed pruritus and erythematous macules and papules scattered all over the body. She was seen in the Emergency Room, and after receiving diphenhydramine hydrochloride (50 mg im), she had partial remission of symptoms. One week later, she had persistent swelling of neck and tongue with mild erythematous macules in the skin, all of which completely disappeared after 1 week of oral prednisone treatment. She was taking sulfisoxazole 3 days before the skin rash episode, and it was assumed that the allergic reactions could have been caused by the sulfisoxazole. On the first day of the sixth melphalan course she took 6 mg of melphalan as a single dose. Within 10 minutes, she had tingling in the hands, dizziness, general pruritus, and a feeling of faintness. Her blood pressure at home was 60/30 mm Hg (lying down). She also had abdominal cramping and mild dyspnea. She was brought immediately to the Emergency Room and was found to have blood pressure of 70/40 mm Hg, with diffuse erythematous macules all over the body. She also had bilateral respiratory wheezes. She was given epinephrine (0.4 ml sc), diphenhydramine hydrochloride (50 mg iv), hydrocortisone (100 mg iv), and saline infusion (iv). Her symptoms subsided completely within a short time. She was given prednisone for 1 week orally. Because she had also taken Dyazide and lorazepam shortly before taking melphalan, the drug which caused the anaphylactic reaction could not be identified. She was advised to discontinue all medication. Melphalan was considered very unlikely to be the cause of her allergic reactions. After 30 days without medication, the patient was given 6 mg of melphalan orally. Within 15 minutes, she started feeling dizzy, noted tingling in the hands, and had palpitations and sweating. Her face swelling was evident within 5 minutes. Her blood pressure dropped from normal to 90/60 mm Hg. She was immediately given diphenhydramine hydrochloride (50 mg im), cortisone, and other supportive measures were taken. All of the symptoms subsided within 24 hours. Since then she has not been given any other alkylating agents. We were concerned that structurally related compounds might produce the same side effects. She has resumed taking Dyazide and lorazepam without any side effects.

DISCUSSION

Hypersensitive reactions like erythema multiforme can occur with mechlorethamine therapy (1). Anaphylactic reaction to iv cyclophosphamide has been reported (2,3). Recently, anaphylactic reactions to iv melphalan have also been observed (4). It would thus appear that the iv administration of alkylating agents can sometimes lead to severe allergic reactions.

In our patient, circumstances clearly indicate that a hypersensitive reaction occurred after oral melphalan therapy. It should be noted that the patient's first anaphylactic reaction to melphalan occurred after only five courses of therapy. Thereafter the reaction developed after each course of therapy. When the patient had taken no other medications, she still developed hypotension and pruri-

tus within 30 minutes of receiving oral melphalan. The hypersensitive reaction was controlled with steroid, antihistamine, and epinephrine therapy. We elected not to administer other alkylating agents. In two previous case reports of hypersensitivity reactions, the authors elected to administer chlorambucil to patients who had previously reacted to mechlorethamine hydrochloride (1) and to cyclophosphamide (5). The chlorambucil did not result in recurrence of the hypersensitive response in either of these cases.

Hypersensitive reactions have been classified into four categories. Type I reaction, otherwise known as anaphylactic reaction, results from the reaction of antigen to reaginic antibody that is fixed to the surface of tissue mast cells or basophils. The result is the release of mediators that have vasoactive and smooth muscle-contracting properties or cellular chemotactic activity, usually within 30 minutes of contact with the incriminating antigen. Anaphylactic reactions due to various oral drugs and foods have been described (5). The mechanism of the type I hypersensitive reaction due to cyclophosphamide is assumed to be IgE-mediated (3) and it is also postulated that cyclophosphamide can act as an immunogenic hapten capable of eliciting an IgE antibody response. It is noteworthy that melphalan has been used as a hapten in the immunogenic treatment of malignant disease (6). In our patient, oral melphalan could have behaved as the hapten which ultimately caused antigen and antibody reaction resulting in release of pharmacologic mediators producing clinical symptoms. Oral ingestion of antigen may precipitate symptoms hours later, although some patients may experience symptoms within minutes as described in our patient.

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Early Detection of Lung Toxicity After Bleomycin Therapy

Bleomycin is a valuable drug in the management of advanced teratoma of the testis. Unfortunately, it can produce lung damage which may progress to fatal pulmonary fibrosis. In such cases it has been reported that the uptake of gallium-67 by the lungs is increased (1). We wish to report a case where gallium-67 uptake increased in the lungs of a symptom-free patient after bleomycin therapy, yet at post-mortem examination 3 months later the lungs showed none of the characteristic abnormalities of bleomycin lung toxicity.

CASE REPORT

A 23-year-old male had massive abdominal disease secondary to teratoma of the testis and was treated with vinblastine and bleomycin with excellent clinical response using the VB_3 regimen of Samuels et al (2). Gallium scans were done on an Elscint WBS-220 double-headed scanner 48 hours after the administration of 3 millicuries of gallium-67 citrate (Radiochemical Centre, Amersham/Searle Corp) (fig 1). The scan done on November 1, 1978 (total bleomycin dose, 300 mg) was normal. By January 4, 1979 (total bleomycin dose, 600 mg), there was widespread uniform uptake of gallium-67 over both lung fields with a negative cardiac shadow. At this time the patient had no respiratory symptoms, and physical examination of the chest was normal, as were chest roentgenogram and pulmonary function tests including transfer factor. Prednisolone at a dose of 40 mg daily was started immediately, and the scan repeated 11 days later showed that the lung fields had returned to normal. Steroids were gradually discontinued after 1 month and a further scan in March 1979 (not shown) remained normal.

Since it was felt that further bleomycin was contraindicated, the patient had uneventful debulking

FIGURE 1.—Anterior views of gallium-67 citrate scans. Normal activity is seen in the liver. Note the diffuse lung uptake in middle scan (4-1-79) with the negative cardiac shadow.

laparotomy on January 25, 1979, under steroid cover. Subsequent treatment included chemotherapy with cyclophosphamide, doxorubicin, *cis*-diamminedichloroplatinum(II), vincristine, and abdominal radiotherapy, but abdominal disease recurred and the patient died on April 18, 1979. Post-mortem examination confirmed the massive abdominal disease recurrences, but showed no evidence of alveolar damage or other features consistent with bleomycin damage in the lung parenchyma (3).

DISCUSSION

Gallium-67 is taken up poorly by normal lungs (4). Diffusely increased uptake of gallium-67 by the lungs has been previously described in association with pulmonary fibrosis following bleomycin therapy (1). In all of these cases, however, there were diffuse hilar opacities on chest roentgenogram, and lung biopsy confirmed the presence of fibrotic changes. Further fibrosis was arrested, and some clinical improvement occurred following high-dose steroid therapy, although the radiologic abnormalities persisted after the gallium-67 scan had returned to normal. It is interesting to note, however, that in the above cases some areas of the lung originally showing an abnormal uptake of gallium-67 were radiologically normal throughout. Diffuse uptake of gallium-67 by the lungs in the presence of normal chest roentgenogram has previously been described following lymphangiography (5) and after chemotherapy with vincristine, cyclophosphamide, and other oncolytic agents, but not after bleomycin (6). At the time our patient's scan became abnormal he had not had lymphangiography and had received only vinblastine and bleomycin. We think that the vinblastine can be excluded as the toxic agent since further courses of the chemically similar vincristine subsequently produced no clinical, scan, or histologic evidence of pulmonary damage.

We believe that this is the first report of an increased uptake of gallium-67 by the lung parenchyma following bleomycin therapy, which was unaccompanied by roentgenographic changes. The finding of completely normal lung parenchyma at post-mortem examination would suggest that these changes either resolved spontaneously or were completely reversed by the prompt administration of high-dose steroids.

It is not possible to state whether the scan changes were the earliest signs of bleomycin lung toxicity or merely represented a nonspecific change to an unknown symptomless stimulus. Because bleomycin is such a valuable drug in the management of advanced testicular cancer a positive gallium-67 lung scan should alert the physician to the possibility of bleomycin lung toxicity, but without biopsy confirmation such a scan may be insufficient reason for discontinuing administration of the drug to a responding patient.

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Pharmacokinetics of ICRF-187 in the Cerebrospinal Fluid of Subhuman Primates

ICRF-187 is an antitumor agent currently undergoing phase I clinical trials in man. ICRF-187 is the enantiomer of ICRF-159. It has been used to increase the solubility of ICRF-159 (1), which is desirable because pharmacokinetic studies have shown that the absorption of orally administered ICRF-159 in man is erratic (2). The enantiomer, ICRF-187, has a solubility of 10 mg/ml in water compared to 3 mg/ml for the racemic form, ICRF-159. The more soluble ICRF-187 can be administered iv.

Since we have recently become involved in phase I trials with ICRF-159, particularly in patients having refractory leukemia with CNS involvement, the question of ICRF-187 penetration into the cerebrospinal fluid (CSF) arose. To determine whether ICRF-187 entered the CSF, the pharmacokinetics of ICRF-187 in the CSF of three adult rhesus monkeys (*Macaca mulatta*) was examined with the use of radiolabeled (^{14}C in the carbonyl position) ICRF-

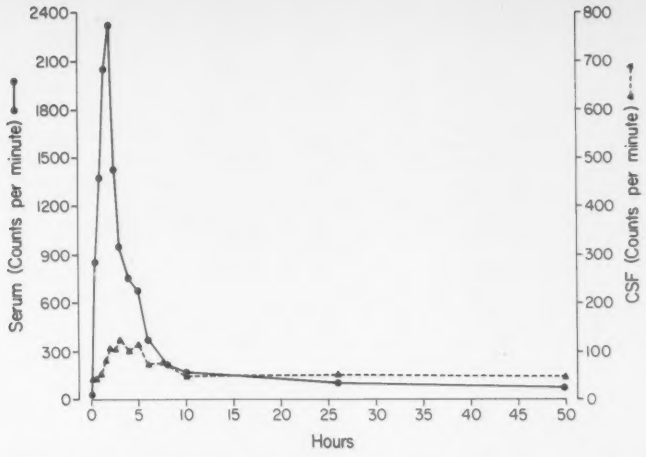

FIGURE 1.—Levels of radioactive ICRF-187 in the blood and CSF of rhesus monkeys vs time after dosing.

187. Radiolabeled ICRF-187 was used because methodologies for measuring cold drug have not yet been developed. All three monkeys had had prior implantation of Ommaya reservoirs according to the method described by Wood et al (3). CSF pharmacokinetics in this model closely parallels CSF drug kinetics in man (4).

Following a 2-hour iv infusion of 300 mg/m^2 (25 mg/kg) of ICRF-187 (toxic dose low in the monkey) spiked with 1.2 mg of ^{14}C-ICRF-187 (37 microcuries/mg), ventricular CSF samples from the Ommaya reservoirs and simultaneous plasma samples were obtained over 48 hours. Plasma was separated, and radioactivity was counted on a Hewlett Packard liquid scintillation counter.

Figure 1 shows the relative levels of radioactivity (counts per minute) in blood and CSF versus time after dosing. Levels of CSF radioactivity reached approximately 10% of the plasma levels of radioactivity near the end of the 2-hour infusion. If we assume that the parent ICRF-187 is not degraded, then peak plasma levels of ICRF-187 in the monkey at the end of the infusion were approximately 9 μg/ml. L1210 leukemia in tissue culture was used in a biologic assay to try to determine if the ICRF-187 which did get into the CSF was cytotoxic. However, the monkey CSF itself effected the growth of L1210 and the cytotoxicity studies could not be done. Twenty-four hours after the infusion, the CSF radioactivity was approximately 50% that of the plasma, but the drug levels would be unlikely to be cytotoxic to leukemia cells. Unfortunately, there is no information available on the level of ICRF-187 needed to produce cytotoxicity in any in vitro system.

From this limited study it appears that very little radiolabeled ICRF-187 crosses into the CSF of rhesus monkeys given 2-hour infusions of the drug. Patients are also receiving 2-hour infusions of ICRF-187 due to the limited solubility of the compound.

ICRF-159 has shown minimal activity in acute lymphocytic leukemia (5,6). Phase II clinical trials are currently attempting to define the level of that activity. Since ICRF-187 is given iv, the bioavailability will likely be better for ICRF-187 than for ICRF-159. This improved bioavailability makes the drug attractive for phase II trials in refractory acute lymphocytic leukemia. From the data presented in this study it seems unlikely that the drug will reach significant levels in the CSF of man.

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ANNOUNCEMENTS

National Cancer Institute Seeking Novel Synthetic Compounds for Anticancer Evaluation

The Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, seeks your participation in the search for new anticancer agents. Compounds contributed by the scientific community continue to be a major source of new leads for anticancer drug development.

We would like to select novel candidate compounds for potential screening as anticancer agents from as large a pool of compounds, and as wide a diversity of structural types, as possible. Structure-activity studies, as well as our extensive experience with the large number of compounds previously screened, allow us to select compounds maximizing structural uniqueness and anticancer potential. It is no longer possible for us to screen all available compounds.

We actively solicit your participation and support in furnishing us a list of compounds for anticancer evaluation. *For further details, please contact either*: Dr. Ken Paull, Chemist, Drug Synthesis and Chemistry Branch, *or* Dr. Ven L. Narayanan, Chief, Drug Synthesis and Chemistry Branch, Blair Bldg, Rm 4A01, 8300 Colesville Rd, Silver Spring, MD 20910.

Neuro-Oncology Fellowships

Applications are being accepted for Neuro-Oncology Fellowship positions for 1981. The purpose of this program is to train neurologists, neurosurgeons, and medical oncologists in therapeutic and clinical neuro-oncology and laboratory research. Neurosurgeons may also participate in the stereotactic interstitial radiation program. One or two years may be elected and stipends are variable depending on previous training. This is an equal opportunity/affirmative action employer. *Send resume and letters of reference to*: Victor Levin, MD, Chief, Neuro-Oncology Service, Brain Tumor Research Center, HSW 783, University of California, San Francisco, CA 94143.

Medical Oncology Fellowships

Applications are being accepted for Medical Oncology positions to start July 1, 1981 and 1982, at the Los Angeles County-University of Southern California Medical Center. Board eligibility in medicine is required. A combined Oncology-Hematology program is available. EOE/MF. *Send CV to*: Malcolm S. Mitchell, MD, Chief, Medical Oncology, Room 10–442, 1200 N State St, Los Angeles, CA 90033.

Carcinogenesis—The New Monthly Journal From IRL

First published in January 1980, *Carcinogenesis* is a multidisciplinary journal designed to bring together all the varied aspects of research which will ultimately lead to the prevention of cancer in man. The journal will publish full papers and short communications which warrant prompt publication in the areas of carcinogenesis; mutagenesis; factors modifying these processes such as DNA repair, genetics and nutrition; metabolism of carcinogens; the mechanism of action of carcinogens and promoting agents; epidemiological studies; and the formation, detection, identification and quantification of environmental carcinogens.

Send contributions to the Executive Editors: Dr. A. Dipple, Frederick Cancer Research Center, P. O. Box B, Frederick, MD 21701, or Dr. R. C. Garner, Cancer Research Unit, University of York, Heslington, York Y01 5DD, UK. *For further information, please write*: Information Retrieval Inc, Fisk Bldg, Suite 815, 250 W 57th St, New York, NY 10019; *telephone*: (212) 245-1438; *telex*: Arlington, Virginia, 899 144.

European Organization for Research on Treatment of Cancer-National Cancer Institute Symposium on Nutrition of the Cancer Patient

Brussels, Belgium, January 8–9, 1981

The European Organization for Research on Treatment of Cancer-National Cancer Institute (EORTC-NCI) will present a symposium on "Nutrition of the Cancer Patient" to be held in Brussels, Belgium, on January 8–9, 1981.

This symposium will deal with several aspects of the cancer patient including anorexia, host-tumor metabolism, nutritional support, and nutrient regulation of tumor growth. The program will include invited presentations and free communications. Attendance will be limited to the first 300 participants. The registration fee is $120.00. *For information contact*: Dr. Daniel L. Kisner, EORTC Data Center, Institut Jules Bordet, 1 rue Héger-Bordet, 1000 Brussels, Belgium (telex: 22773; phone: 538.57.90).

Current Thinking in the Management of Ocular and Adnexal Tumors

San Francisco, California, February 4–6, 1981

This course will focus on the practical aspects in the diagnosis and management of lid, conjunctival, orbital, and intraocular tumors. New advances in diagnosis and therapy will be stressed; expert round-table discussions will augment the lectures.

Subjects to be discussed include: masquerade syndromes, lid and conjunctival tumor management, controversies in the management of choroidal melanoma, iris tumors and ocular metastases, retinoblastoma, and advances in orbital tumor diagnosis and management. Guest speakers will include: F. T. Fraunfelder, John Henderson, Jerry Shields, William Spencer, and Lorenz Zimmerman. The UCSF faculty will include: Crowell Beard, Devron Char, William F. Hoyt, G. Richard O'Connor, and Phillips Thygeson.

For further information, please contact: Extended Programs in Medical Education, (415) 666-4251.

Sixth Annual Symposium on Advances in Cancer Treatment Research

Baltimore, Maryland, March 12–14, 1981

The program of Continuing Education, University of Maryland School of Medicine is presenting its Sixth Annual Symposium on Advances in Cancer Treatment Research on March 12–14, 1981, at the Baltimore Convention Center in Baltimore, Maryland. *For additional information contact*: The Program of Continuing Education, University of Maryland School of Medicine, at 301–528-3956.

Third International Conference on the Adjuvant Therapy of Cancer

Tucson, Arizona, March 18–21, 1981

The Third International Conference on the Adjuvant Therapy of Cancer will be held at the Tucson Convention Center, March 18–21, 1981, under the sponsorship of the Cancer Center Division of the University of Arizona. Drs. Sydney E. Salmon and Stephen E. Jones will serve as Conference Co-Chairmen. *For further information, please contact*: Mary Humphrey, Conference Coordinator, Cancer Center Division, University of Arizona, Tucson, AZ 85724.

Latin American Integrated Cancer Congresses

São Paulo, Brazil, May 10–15, 1981

This meeting will include the 7th Latin American Cancer Congress, the 7th Latin American Chemotherapy Congress and several other regional meetings. The

program provides round-tables, informal discussions, lectures by invited speakers, and free communications. Special emphasis will be given to diagnostic and treatment procedures. The official languages will be Spanish, Portuguese, and English.

The Congress is sponsored by "Fundação Antonio Prudente" with Dr. A. C. C. Junqueira (President), Dr. F. Gentil (Chairman, Scientific Committee), Dr. A. Abrão (Chairman, Organizing Committee), and Dr. A. P. Mirra (Secretary General). Registration fee is $100.00 before January 31, 1981, and $120.00 after January 31, 1981. *For more information contact*: The Secretariat, Hospital A. C. Camargo, Rua Professor Antonio Prudente, 211, Caixa Postal (P. O. Box) 5271, São Paulo, Brazil.

International Conference on Malignant Lymphomas: Current Status and Prospects

Lugano, Switzerland, September 2–5, 1981

On September 2–5, 1981, the International Conference on Malignant Lymphomas: Current Status and Prospects will be held in Lugano, Switzerland. The Organizing Committee is G. Bonadonna (Milan, Italy), F. Cavalli (Bellinzona, Switzerland), and M. Rozencweig (Brussels, Belgium).

Despite major progress recently achieved in the prognosis of malignant lymphomas, there is a continuous need for improving therapeutic results in these diseases. This conference will deal with multidisciplinary approaches in the treatment of Hodgkin's and non-Hodgkin's lymphomas in children and adults. Topics to be covered include: Hodgkin's Disease (prognostic factors and subgroups, modern staging and treatment, long-term toxicity) and Non-Hodgkin's Lymphomas (kinetic behavior, biologic markers, new attempts at a unified pathologic and clinical classification, staging and restaging, standard and newer therapies).

Most lectures will be given by invited speakers. Selected controversial issues will be discussed by panels of leading experts. The program will include a limited number of free presentations. A poster session will also be organized.

For further information, write to: F. Cavalli, Head, Division of Oncology, Ospedale San Giovanni, CH-6500 Bellinzona, Switzerland.

International Course on Human Immunology and Cancer Immunomodulation—A Two-Day Intensive Course

Paris, France, January 15–16, 1981

On January 15–16, 1981, there will be an International Course on Human Immunology and Cancer Immunomodulation, to be held in Paris, France. The first day will be devoted to the phenotype of NK cells in mice and humans, and the nature of effector-target cell interaction and specificity. The second day will be devoted to the regulation of NK cell activity, and the in vivo relevance of NK cells.

The organizers of the course are Dr. B. Serrou (Montpellier, France) and Dr. C. Rosenfeld (Villejuif, France). The invited organizer and chairman is Dr. R. Herberman (Bethesda, MD, USA). Invited speakers include: Dr. Cudkowicz (USA), Dr. Herberman (USA), Dr. E. Klein (Sweden), Dr. Kiessling (Sweden), Dr. Ortaldo (USA), Dr. Riccardi (Italy), Dr. Roder (Canada), Dr. Saksela (Finland), Dr. Warner (USA), and Dr. Wigzell (Sweden).

The registration fee is 750 French francs. *For further information, please contact*: Dr. Serrou, Laboratoire d'Immunopharmacologie des Tumeurs, Centre Paul Lamarque, Hôpital St-Elio, B.P. 5054, 34033 Montpellier, Cédex, France.

International Symposium on Nitrosoureas in Cancer Treatment

Montpellier, France, January 22–23, 1981

On January 22–23, 1981, there will be an International Symposium on Nitrosoureas in Cancer Treatment, to be held at the Sofitel Hotel, "Le Triangle,"

Montpellier, France. There will be two sessions, 1) Chemistry Mechanism of Action and Experimental Evaluation, and 2) Clinical Evaluation.

Individual communications (10 minutes) will be presented at the meeting. Abstracts must be submitted in English (1 original and 2 xerox copies) before December 1, 1980.

The registration fee is 750 French francs. Please make check payable to "International Symposium." Please indicate name, university, or company affiliation, address, and whether an abstract is to be submitted. The check must be enclosed with the registration information. ***Please send registration information, abstracts, and check to the following***: Secrétariat du Symposium International, "Nitrosoureas in Cancer Treatment," Laboratoire de Chimie Bio-Organique, Université des Sciences et Techniques du Languedoc, Place Eugène Bataillon, 34060 Montpellier, Cédex, France (Telephone: 63.91.44 ext. 849).

Annual Meeting of the Cell Kinetics Society

Asilomar, California, March 1–4, 1981

The Fifth Annual Meeting of the Cell Kinetics Society will be held in Asilomar, California, on March 1–4, 1981. This will be a joint meeting with the Annual Workshop on Biomathematics and Cell Kinetics.

The Cell Kinetics Society has an international membership of approximately 400 experimentalists, clinicians, and mathematical modelers who share an interest in the cell proliferation patterns of normal and abnormal tissues.

Inquiries on the meeting or membership should be sent to: Dr. Eleanor E. Deschner, Secretary, Cell Kinetics Society, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

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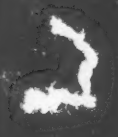
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